UNIVERSITÉ PARIS-SUD

ÉCOLE DOCTORALE 145 :
SCIENCES DU VÉGÉTAL

Laboratoire : *Ecologie, Systématique et Evolution*

THÈSE DE DOCTORAT

BIOLOGIE

par

Alice DELAPORTE

Vers une compréhension fonctionnelle des dépérissements forestiers : étude du cas du hêtre (*Fagus sylvatica* L.) en forêt de Fontainebleau

Date de soutenance : 22/09/2015

Composition du jury :

Directeur de thèse :	Claire DAMESIN	Professeur (Université Paris Sud)
Co-directeur de thèse :	Stéphane BAZOT	Maitre de conférences (Université Paris Sud)
Rapporteurs :	Hervé COCHARD	Directeur de recherche (INRA Clermont-Ferrand)
	Jean-Marc GUEHL	Directeur de recherche (INRA Champenoux)
Examinateurs :	Jean-Marc LIMOUSIN	Chargé de recherche CNRS (Montpellier)
	Nathalie FRASCARIA-LACOSTE	Professeur (AgroParisTech)

Remerciements

Ce mémoire est l'aboutissement d'un peu plus de quatre années de travail au sein de l'équipe écophysiologie végétale, au laboratoire Ecologie, Systématique et Evolution. Ces années resteront pour moi d'excellents souvenirs.

Je tiens à remercier en premier lieu mes encadrants, Claire Damesin et Stéphane Bazot, qui depuis mon stage de première année de master m'ont transmis leurs connaissances, formée et encouragée. Merci de m'avoir confié ce projet de recherche.

Merci aux membres du jury d'avoir accepté d'évaluer ce travail.

Merci également aux membres de mon comité de thèse de m'avoir apporté leur avis éclairé vers le milieu de ma thèse : Stéphane Herbette, Pascale Maillard et Jean-Christophe Lata.

Au cours de ces quatre dernières années, de nombreuses personnes ont contribué à ce travail par leur collaboration scientifique :

- Chantal Fresneau, qui m'a transmis ses connaissances en matière de dosages de sucres, amidon et protéines, et plus généralement de tout ce qui peut être utile quand on travaille dans un labo : minutie, rigueur, méthode, et petits bricolages

- Eric Dufrêne, qui m'a appris à quantifier le débourrement et le jaunissement à mes débuts (et à m'occuper des plantes carnivores)

- Anne-Violette Lavoir et Anaïs Boura pour la quantification des cellules vivantes

- Stéphane Herbette pour son aide précieuse lors des mesures de vulnérabilité à la cavitation

- Romain Joseph et Têté Barigah pour les subtilités des mesures au Xyl'Em

- Thierry Améglio pour les mesures de résistance à la congélation

- Philippe Rozenberg pour les données de microdensité

- Monique Pierre, Valérie Daux et Florent Barbecot pour le projet finalement non abouti de mesures de $\delta^{18}O$

- Yves Gibon de l'unité Biologie du Fruit et Pathologie de l'INRA de Bordeaux pour les dosages d'acides aminés

- Joannès Guillemot et Gabriel Hmimina pour leur aide sur l'analyse de certaines données particulièrement complexes

Je remercie également les personnes qui m'ont apporté une aide technique :

- Alain Sévéré (« chouquette-man ») et Jean-Louis Mabout pour leur aide sur le terrain, et tout spécialement pour les tirs de branches au fusil (beaucoup plus compliqué que ça n'en n'a l'air…)

- Michèle Viel et Patricia Le Thuaut pour les innombrables pré-broyages et broyages d'échantillons, toujours avec énergie et bonne humeur

- sans oublier les stagiaires, dans l'ordre chronologique : Cédric Laborie, Hélène Guerreiro, Jean-Charles Miquel, Laura Flamigni, Simon Legendre, Ludivine Lhotelin et Mathilde Bugeat.

Plus généralement, merci à toute l'équipe Ecophysiologie végétale, pour les discussions scientifiques, moins scientifiques, voire franchement loufoques, et les bons moments partagés. Je souhaite à tout doctorant de pouvoir travailler dans une équipe aussi chaleureuse, soudée, et excellente scientifiquement. Merci à Nicolas pour les trois années de cohabitation dans un bureau commun, en dépit de nos optimums thermiques radicalement différents. Merci à Zoran et Gwendal de m'avoir accueillie dans leur bureau pour les derniers mois, ainsi que pour les patientes leçons de serbo-croate de maitre Z. Merci à Gaëlle, Chantal et Nathalie qui m'ont plus d'une fois remonté le moral, particulièrement pendant la dernière année.

Un merci spécial à mes collègues et ex-collègues doctorants et post-doctorants : Lydie Blottière, Joannès Guillemot, Elodie Merlier, Gabriel Hmimina, Elena Granda, Marc Stéphanon, Marine Pasturel, Guillaume Marie, Alice Michelot, Angélique Gilson, Constance Laureau et Sébastien Bellow. Lydie, Elodie : courage les filles, vous y êtes presque !

Merci aux hêtres 1S, 6S, 9S, 14S, 16S, 107D, 109D, 110D, 112D et 113D, qui ont légèrement souffert pour la science.

Je tiens enfin à remercier ma famille, qui m'a pendant toutes mes études soutenue et encouragée. Bon courage à mon frère, qui ne s'est pas laissé effrayer par l'état de sa vieille sœur en fin de thèse, et qui commencera la sienne à l'automne.

Sommaire

I. Introduction

1. Les forêts face aux changements climatiques

Les écosystèmes forestiers recouvrent environ 31% des surfaces émergées (Lindquist et al. 2012), et 30% du territoire français (Inventaire Forestier National (IFN) 2010). Les forêts présentent de nombreux intérêts, par exemple économiques via la production de bois (charpente, menuiserie…) et de produits dérivés (papiers, granules…). Leur rôle dans la séquestration de carbone atmosphérique leur confère en particulier une importance majeure dans la mitigation des changements globaux, notamment du réchauffement climatique. Dans le cas des forêts tempérées, les changements globaux, via entre autres une augmentation de la température, de la concentration en CO_2 atmosphérique et d'une fertilisation azotée, ont (au moins à moyen terme) un impact positif sur la croissance des arbres, notamment grâce à un allongement de la saison de végétation (Lindner et al. 2010).

Dans les prochaines décennies, les changements climatiques vont en revanche engendrer des modifications des aires de répartition des espèces de l'ordre de dizaines, voire de centaines de kilomètres vers les pôles (Intergovernmental Panel on Climate Change 2014). Grâce à une approche multi-modèles, Cheaib et al. (2012) montrent qu'une forte contraction des aires de répartition est à attendre en plaine pour plusieurs espèces d'arbres majeures des forêts européennes (dont le hêtre). Ces modifications pourraient avoir d'importants impacts écologiques, économiques et sociaux, particulièrement dans les cas où elles s'accompagneraient d'une forte augmentation de la mortalité des arbres (Lindner et al. 2010). Dans le cas des forêts tempérées, en parallèle de l'allongement de la saison de végétation, il est en effet prévu une augmentation de la fréquence et de l'intensité des événements extrêmes, comme par exemple des sécheresses (Intergovernmental Panel on Climate Change 2014). L'impact négatif de cette augmentation des stress ponctuels pourrait surpasser l'impact positif de l'allongement de la saison de végétation, notamment dans les zones où les conditions hydriques sont déjà limitantes (Lindner et al. 2010). Les populations d'arbres poussant dans

des conditions défavorables ont en effet une vulnérabilité accrue à ces stress ponctuels (Bréda and Badeau 2008), ce qui peut se traduire localement par des phénomènes de dépérissement et/ou de mortalité. Des dépérissements forestiers sont signalés dans tous les biomes forestiers du monde (Allen et al. 2010). Le nombre de cas de dépérissements forestiers rapportés dans la littérature à fortement augmenté durant la dernière décennie, sans qu'il soit pour l'instant possible de dire si cette augmentation reflète une réalité de terrain, ou si elle reflète simplement un plus grand intérêt de la communauté scientifique pour le sujet (Allen et al. 2010). Quoi qu'il en soit, de tels événements entraînent pour les exploitants une perte financière importante (soit car les arbres sont trop jeunes pour être exploités, soit par perte de la qualité du bois suite aux dommages subis) (Mormiche 1994). Les espèces concernées par le dépérissement sont parfois des essences à forte valeur économique (par exemple des chênes ou des hêtres en milieu tempéré). Une baisse de qualité du bois contribue par conséquent à accroître les inquiétudes engendrées par ces événements.

2. Les dépérissements forestiers

2.1. Définition

Le terme de dépérissement peut s'utiliser soit à l'échelle de la population d'arbres (massif forestier, parcelle…), soit à l'échelle individuelle. A l'échelle d'une population, un dépérissement est défini comme une perte de vigueur constatée sur de nombreux arbres vivant dans des conditions communes (Mueller-Dombois 1988). A l'échelle individuelle, le dépérissement s'exprime visuellement par un ensemble de symptômes, principalement visibles au niveau du houppier (augmentation de la transparence, Figure I.1), mais également au niveau du tronc (suintements, lésions en bande, champignons sur les blessures) et des racines (présence d'un mycélium sous-cortical et de rhizomorphes) (Nageleisen 1994). Il est important de noter que ces symptômes peuvent être réversibles, le dépérissements ne se

soldant pas nécessairement par la mort de l'arbre (Landmann 1994). Les symptômes de dépérissement peuvent dans certains cas évoquer ceux d'une sénescence naturelle. La distinction entre un dépérissement et un phénomène normal de sénescence se fait alors sur l'âge auquel se déclarent les symptômes : on parlera de dépérissement s'ils interviennent de façon prématurée dans la vie de l'arbre.

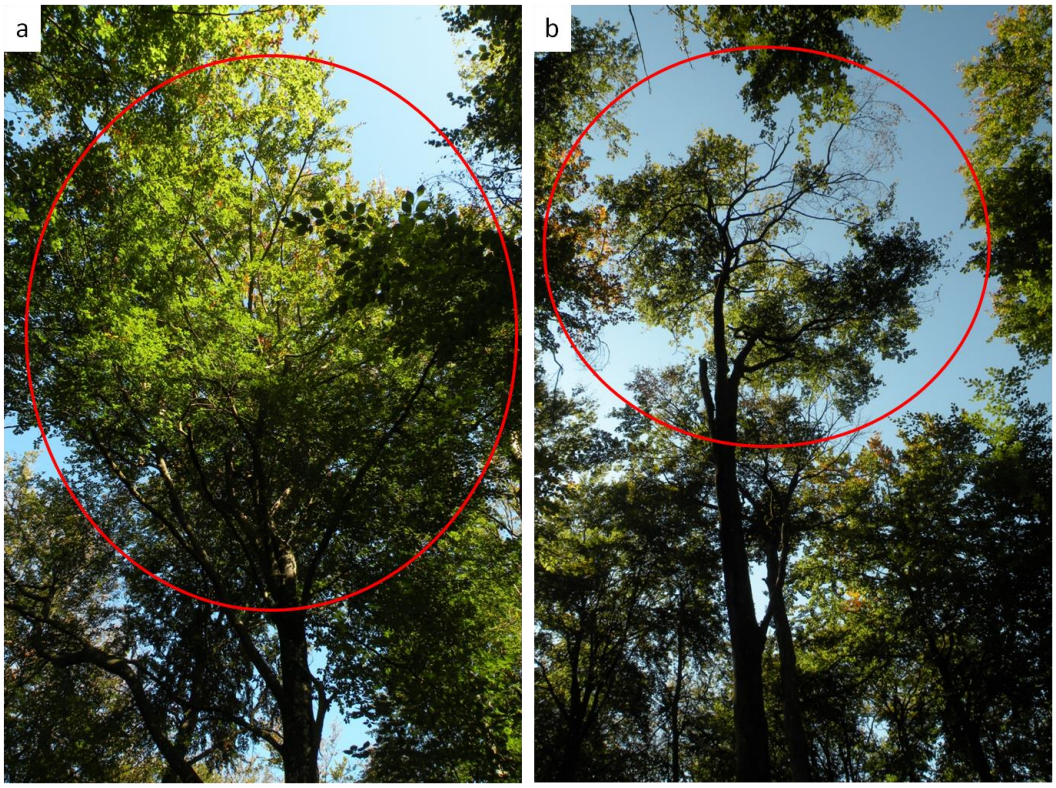

Figure I.1 Houppiers d'un hêtre sain (a) et d'un hêtre dépérissant (b), en forêt domaniale de Fontainebleau. Source: photos personnelles.

En France, l'estimation de ces symptômes est normalisée par l'utilisation du protocole DEPEFEU, mis au point par le Département de Santé Forêt. Ce protocole permet l'évaluation de l'état de houppiers de feuillus et de conifères sur plusieurs critères. Les principaux sont la transparence du houppier, la mortalité d'organes pérennes (branches) et la répartition de la masse foliaire. Une note, de 0 (arbre sain) à 4 (arbre mort ou moribond) est alors attribuée à l'arbre (Nageleisen and Goudet 2011). Cette évaluation, en association avec le suivi

systématique des forêts effectué par l'IFN (Inventaire Forestier National), permet d'obtenir des données sur le dépérissement des principales espèces forestières à l'échelle de la France. Par exemple, la majorité des dépérissements de hêtres actuels sont signalés dans le nord-est de la France (Nageleisen and Reuter 2007).

Les phénomènes de dépérissement, correspondant à des détériorations de houppier sur plusieurs années, sont à distinguer des phénomènes de mortalité, d'évolution plus rapide (un an ou deux). Dans la suite de ce travail, les termes dépérissement et mortalité désigneront donc des réalités différentes.

2.2. Un bref historique

Les premiers dépérissements forestiers répertoriés sont datés du début du XX^{ème} siècle. Ils se sont amplifiés au cours de la seconde moitié du XX^{ème} siècle, entrainant un fort intérêt pour le sujet (Landmann 1994). A partir des années 1970, d'importants dépérissements sont observés notamment en Allemagne, sur le sapin pectiné (*Abies alba* Mill.). Le premier cas de dépérissement en France est décrit en 1982, sur une forêt de sapins pectinés, dans les Vosges (Lévy and Becker 1987). Au début des années 1980, le dépérissement s'étend aux espèces feuillues (hêtre et chênes) (Krause et al. 1986). Cette vague de dépérissement se ralentit, puis se résout vers le milieu des années 1980 (Vennetier 2012). A partir du milieu des années 1990, une nouvelle vague de dépérissements commence à être observée (Nageleisen and Reuter 2007). Ainsi, dans le massif de Fontainebleau, des dépérissements de chênes pédonculés et de hêtres sont notés à partir de la fin des années 1990 (Gilles Defour, communication personnelle).

2.3. Causes externes

Dans son usage courant, le terme « dépérissement » désigne des problèmes de santé des arbres dont la cause est inconnue, ou qui sont causés par plusieurs facteurs (Krause et al. 1986; Sinclair and Hudler 1988). Quand ils sont identifiés, les facteurs liés aux dépérissements peuvent être classés en trois catégories : des facteurs prédisposants, des facteurs déclenchants, et des facteurs aggravants (Manion 1981; Sinclair and Hudler 1988). Manion (1981) représente ces facteurs sous la forme d'une spirale (Figure I.2), dans laquelle un même facteur (par exemple la sécheresse) peut appartenir à plusieurs catégories (prédisposant, déclenchant ou aggravant) selon sa durée et son intensité.

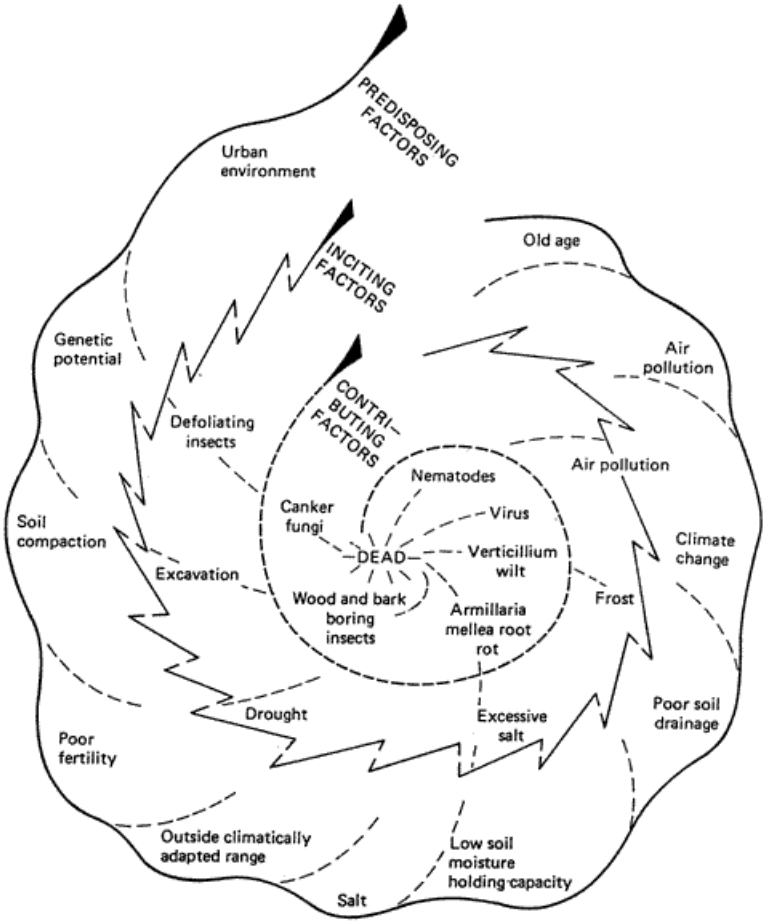

Figure I.2 La spirale du dépérissement, illustrant les interactions entre facteurs prédisposants, déclenchants et aggravants. Illustration tirée de Manion (1981).

Lors de la vague de dépérissement des années 1980, en Europe et en Amérique du Nord, les pluies et dépôts acides ont été pointés comme une des causes majeures du dépérissement (Tomlinson 1983; Godbold et al. 1988). Les dépôts acides perturbent notamment l'équilibre chimique des sols, conduisant à un déficit en ions facilement échangeables (Mg^{2+}, Ca^{2+}, K^{+}) et à une surcharge de la solution du sol en ions Al^{3+}, toxiques pour les plantes (Godbold et al. 1988). Il a en revanche été rapidement démontré que la pollution atmosphérique n'était pas l'unique cause des dépérissements (Johnson and Siccama 1983). En particulier, le rôle de sécheresses chroniques, ou d'événements ponctuels de sécheresses extrêmes dans le déclenchement des dépérissements forestiers a été mis en évidence (Johnson and Siccama 1983; Becker and Levy 1988).

En ce qui concerne la vague de dépérissement actuelle, il semble probable que les changements climatiques aient un rôle, via une augmentation de la fréquence et de l'intensité des événements extrêmes (vagues de sécheresse ou de canicule par exemple) et/ou en modifiant les aires de répartition et les dynamiques démographiques de certains ravageurs (Allen et al. 2010).

2.4.Cas d'un dépérissement de hêtres à Fontainebleau

2.4.1. La forêt domaniale de Fontainebleau

La forêt domaniale de Fontainebleau est une forêt tempérée, située à 60 km au Sud-est de Paris (48°25 N, 2°40 E, 120 m d'altitude). Elle s'étend sur 17000 ha divisés en 748 parcelles d'une surface moyenne de 22 ha. Le climat de la région est de type océanique. Pour la période 1960-2010, la température moyenne annuelle est de 10,6°C, et le cumul annuel moyen de précipitations est de 750 mm en moyenne, bien réparti au cours de l'année. La géologie du site est complexe, avec une couche de sables de Fontainebleau, avec des grès et parfois une couche de calcaire stampien (souvent érodée), auxquelles est superposée une couche de sables éoliens plus ou moins mélangés à des limons des plateaux. De cette géologie complexe résultent de très grandes diversité et variabilité spatiales des sols.

La forêt est gérée par l'Office National des Forêts (ONF). Les principales espèce du peuplement sont le Chêne sessile (*Quercus petraea* (Matt.) Liebl.), le Chêne pédonculé (*Quercus robur* L.), le Hêtre (*Fagus sylvatica* L.) et le Pin sylvestre (*Pinus sylvestris* L.).

2.4.2. Le hêtre (Fagus sylvatica L.)

Le hêtre (*Fagus sylvatica* L.) est une espèce très fréquente dans les forêts françaises : les hêtraies recouvrent environ 9% de la surface des forêts de production. Il s'agit du deuxième peuplement forestier en surface en France (après les chênaies) (Inventaire Forestier National (IFN) 2010). Le hêtre nécessite pour se développer une forte hygrométrie de l'air et une pluviométrie supérieure à 600 mm par an, bien répartie dans l'année. Il tolère de nombreux types de sols, à l'exception des sols très acides ou hydromorphes (Bastien 2000). Le hêtre est une essence d'ombre en milieu mésophile à sec, mais peut se comporter comme une essence de lumière en milieu humide (Teissier du Cros et al. 1981). En l'absence d'action de sylviculture visant à limiter son expansion, le hêtre a tendance à former des futaies denses, quasiment monospécifiques. Le couvert de ces peuplements est alors très dense, et l'éclairement relatif au sol est très faible (environ 4%) (Bastien 2000). Le hêtre présente la particularité de former deux types de rameaux au cours de la saison de végétation : des rameaux d'exploration (longs, avec des bourgeons latéraux bien développés) et des rameaux d'exploitation (courts, souvent sans bourgeons latéraux). Les rameaux d'exploitation, malgré leur petite taille, portent la majorité des feuilles de l'arbre. Ces deux types de rameau reflètent deux stratégies d'occupation du milieu au sein d'un même arbre (Teissier du Cros et al. 1981).

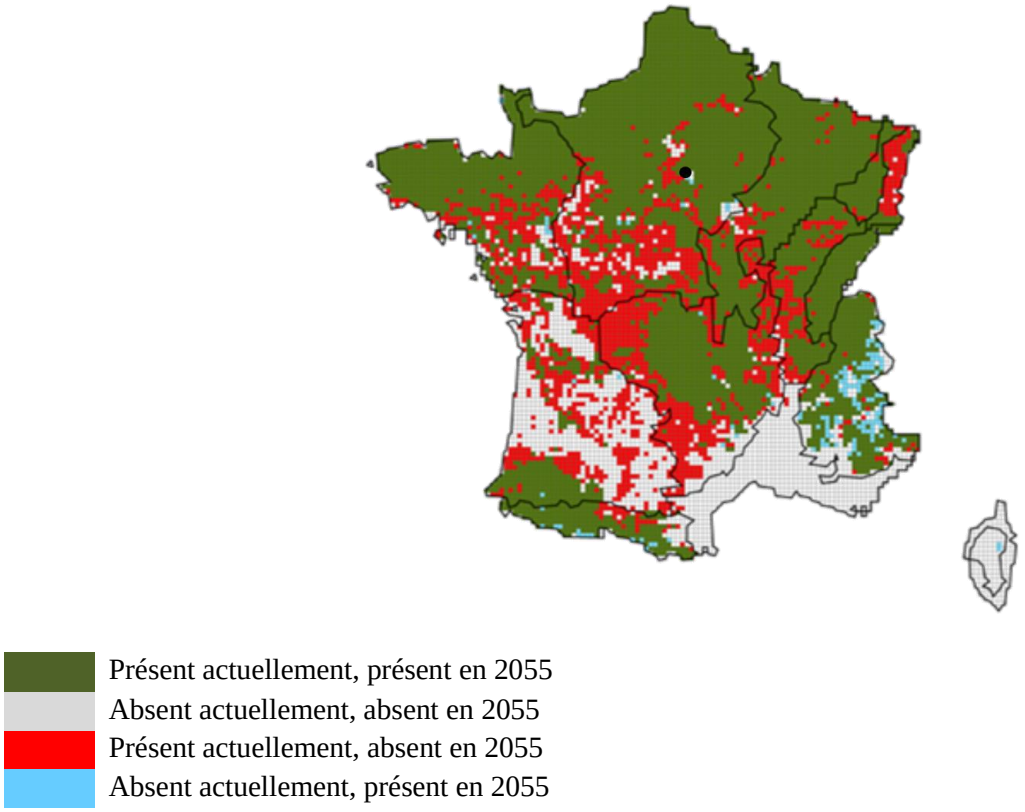

Figure I.3 Aire de répartition du hêtre en 2055 en comparaison avec l'actuelle, telles que prédites par le modèle CASTANEA (Dufrêne et al. 2005). Les projections climatiques sont basées sur le scénario A1B SRES d'émissions de CO_2 de l'IPCC, en utilisant le modèle ARPEGE AGCM de Météo France (Déqué 2007). La position approximative du massif de Fontainebleau est indiquée par un point noir.
D'après Cheaib et al. (2012).

En France, le hêtre est présent en plaine dans la moitié nord et en montagne dans la moitié sud, jusqu'à 1700 m. d'altitude. Des approches de modélisation ont montré qu'au cours des décennies à venir, son aire de répartition risque de se contracter vers le nord (Badeau 2007; Cheaib et al. 2012). Ainsi, en 2055, le hêtre en forêt de Fontainebleau devrait se retrouver en limite de son aire de répartition (Figure I.3, Badeau 2007; Cheaib et al. 2012). Depuis la fin des années 1990, certaines parcelles de hêtre en forêt de Fontainebleau montrent des signes de dépérissements (Defour, ONF, communication personnelle). Les parcelles les plus affectées sont celles qui poussent sur les sols les moins profonds, ce qui est associé à une réserve utile maximale plus faible (Silva 2010).

2.4.3. Caractéristiques et intérêt de la parcelle étudiée

La parcelle d'étude (n° 639) est un peuplement monospécifique de hêtres matures. L'âge moyen est de 95 ans et la hauteur dominante de 27 m. Le sol présente une forte variabilité spatiale, allant d'un sol brun lessivé à un podzol selon les zones. La texture est sableuse, avec une proportion variable de limons et d'argile en fonction de la profondeur. Le ratio C/N est de 18.7/1, et la réserve utile est faible (101 mm). Le site fait partie des premières parcelles à avoir présenté des symptômes de dépérissement, à la fin des années 1990.

Une étude dendrochronologique sur des arbres sains et dépérissants de cette parcelle a révélé une diminution de la croissance radiale des arbres dépérissants à très long terme (Figure I.4, Silva 2010, Michelot 2011). Jusqu'en 1959, les arbres sains et dépérissants ont une croissance radiale similaire. Cependant, dès l'année 1959 (année de sécheresse), les dépérissants présentent une croissance radiale significativement plus faible que celle des sains en l'absence de tout symptôme au niveau du houppier. Les premiers symptômes visuels du dépérissement n'apparaitront qu'une trentaine d'années plus tard, suite à la sécheresse de l'année 1996. Il est à noter qu'aucune attaque notable d'insectes ou de pathogènes n'a été signalée entre 1959 et aujourd'hui (Defour, ONF, communication personnelle).

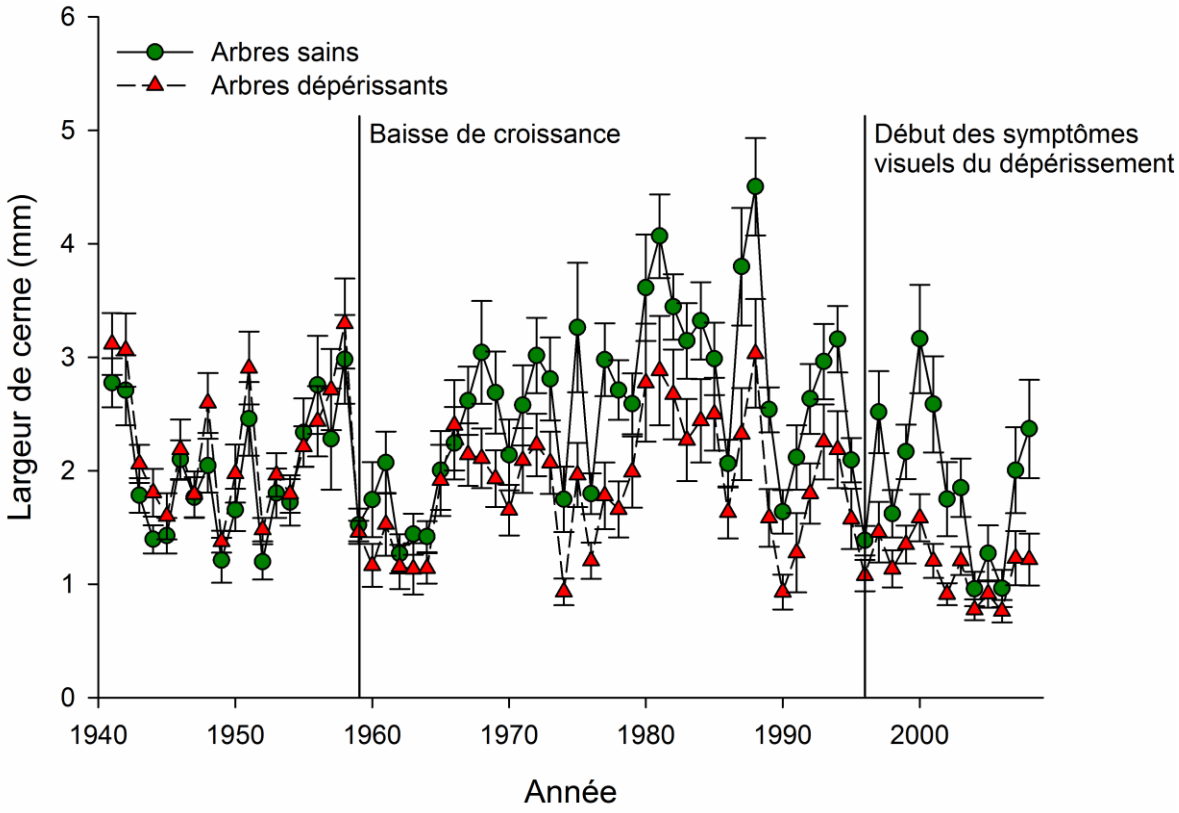

Figure I.4 Largeurs de cernes de hêtres sains et dépérissants poussant en forêt de Fontainebleau (France), de 1930 à 2010. Les barres d'erreur sont des erreurs standard. D'après Michelot (2011) et Silva (2010).

Le site d'étude présente donc un dépérissement à long terme. Les deux moments-clefs dans l'histoire de ce dépérissement -le début de la diminution de croissance, et le déclenchement des symptômes visuels- correspondent par ailleurs à des événements de sécheresse. Il semble donc que des sécheresses répétées fassent partie dans notre cas des facteurs déclenchants (*sensu* Manion 1981). De plus, des conditions édaphiques défavorables (faible réserve utile) ont été associées aux dépérissements de hêtres à Fontainebleau (Silva 2010). Le dépérissement observé est donc probablement un arrière effet à long terme de stress hydriques répétés aggravés par une faible disponibilité en eau du sol. Ce site est de ce fait particulièrement adapté pour étudier les mécanismes fonctionnels sous-jacents à un dépérissement forestier se déroulant sur le long terme.

3. Mécanismes fonctionnels sous-jacents

Du fait du rôle du changement climatique dans la vague actuelle de dépérissement, les mécanismes fonctionnels sous-jacents aux événements de dépérissement et de mortalité sont principalement étudiés dans des contextes de sécheresses extrêmes et/ou chroniques dans la littérature récente. Les hypothèses avancées dans ce cadre sont néanmoins pertinentes pour appréhender la réponse de plantes à des stress de manière plus générale, comme l'illustrent quelques études sur l'effet de défoliations (Wiley et al. 2013; Saffell et al. 2014; Jacquet et al. 2014). La suite du propos sera consacrée principalement aux réponses fonctionnelles des arbres lors de sécheresses, expérimentales ou naturelles, suivies ou non d'une dégradation de l'état des houppiers ou de la mort des arbres.

Deux grandes hypothèses de dysfonctionnement d'un arbre au cours d'une sécheresse engendrant un dépérissement ou une mortalité ont été formulées : une défaillance hydraulique (Martínez-Vilalta et al. 2002; McDowell et al. 2008) et une déficience en réserves carbonées (Waring 1987; McDowell et al. 2008). Ces deux hypothèses ne sont pas mutuellement exclusives. Elles n'ont pas non plus prétention à être exhaustives : d'autres hypothèses de dysfonctionnement, par exemple impliquant des problèmes de nutrition minérale (azote, calcium, magnésium…), peuvent être avancées (Becker and Levy 1988; Godbold et al. 1988).

3.1. Hypothèse hydraulique

Dans le xylème, l'eau liquide circule sous tension, dans un état métastable (Dixon and Joly 1984; Tyree and Sperry 1989). Lors d'une sécheresse, la tension de la colonne d'eau dans le xylème augmente. Si la tension de la colonne d'eau dans le xylème devient trop importante, il se produit un phénomène de cavitation : l'eau passe de l'état liquide à l'état de vapeur (Cruiziat et al. 2002). La présence d'embolie réduit la capacité du xylème à transporter l'eau des racines vers les feuilles. Si une trop grande proportion de vaisseaux de xylème est

embolisée, l'arbre peut se trouver dans l'impossibilité de répondre à la demande transpiratoire du houppier, qui risque alors de se dessécher (McDowell et al. 2008). Les arbres peuvent mettre en place des stratégies pour limiter la formation d'embolie lors d'une sécheresse, notamment en régulant la demande transpiratoire (Bréda et al. 2006). On peut citer comme mécanismes de régulation la fermeture des stomates (Sperry 2000), ainsi que la chute d'une partie du feuillage en vue de diminuer la surface transpirante (Bréda et al. 2006). La défaillance du système de transport d'eau est donc un des mécanismes potentiels pouvant mener à la mort d'un arbre en conditions de sécheresse (McDowell et al. 2008). Plusieurs éléments dans la littérature suggèrent qu'un dysfonctionnement du système hydraulique pourrait avoir un rôle dans la mort des arbres.

Premièrement, la vulnérabilité du xylème d'une espèce à la cavitation est liée au climat et/ou aux conditions édaphiques dans son aire de répartition géographique (Kavanagh et al. 1999; Brodribb and Hill 1999; Pockman and Sperry 2000; Tissier et al. 2004; Poot and Veneklaas 2012; Choat et al. 2012). De plus, à l'échelle mondiale, la vulnérabilité à la cavitation est liée à la quantité de précipitations annuelles chez les gymnospermes et les angiospermes sempervirentes (Maherali et al. 2004). Ces données suggèrent qu'à l'échelle interspécifique, la résistance à la cavitation pourrait être un facteur clef pour comprendre les divers degrés de tolérance des espèces aux sécheresses. En revanche, à l'échelle intraspécifique, Wortemann et al. (2011) ne trouvent pas d'association entre quantité de précipitations annuelles et résistance à la cavitation chez le hêtre.

Un deuxième élément en faveur de l'implication de facteurs hydrauliques dans les événements de mortalités liés aux sécheresses est le lien observé entre la résistance du xylème à la cavitation et le taux de survie lors de sécheresses. Cette relation a été montrée aussi bien sur de jeunes plants (Pratt et al. 2008; Brodribb et al. 2010) que sur des arbres adultes (Cochard et al. 2008; Urli et al. 2013; Nardini et al. 2013). Elle semble valable aussi bien

entre des espèces phylogénétiquement éloignées (Urli et al. 2013; Nardini et al. 2013) qu'au sein d'une même famille (Pratt et al. 2008), ou d'un même genre (Van der Willigen and Pammenter 1998; Cochard et al. 2008; Brodribb et al. 2010).

De manière plus directe, un lien a été montré entre des fortes tensions et/ou de forts taux d'embolie dans le xylème et la mortalité à l'échelle de l'organe ou de l'individu entier. Ainsi, chez un peuplier nord-américain, les potentiels hydriques minimum des branches d'arbres soumis à une sécheresse expérimentale sont plus faibles, et les pertes de conductivité hydraulique plus fortes que ceux des arbres non stressés (Anderegg et al. 2012). Dans la même étude, mais cette fois sur des arbres matures soumis à une sécheresse naturelle, de plus fortes pertes de conductivité hydraulique sont mesurées chez les arbres dépérissants que chez les arbres sains (Anderegg et al. 2012). Dans une autre étude sur six espèces d'angiospermes matures en conditions de sécheresse extrême naturelle, Nardini et al. (2013) observent également que les arbres qui présentent de forts pourcentages de branches desséchées suite à une sécheresse exceptionnelle souffrent de plus grandes pertes de conductivité hydraulique dans les jeunes rameaux que les arbres non atteints. Enfin, sur de jeunes arbres soumis à des conditions expérimentales de sécheresse extrême, la mort de l'arbre a lieu lorsqu'un certain niveau de perte de conductivité hydraulique (autours de 90% chez les angiospermes) est atteint (Urli et al. 2013; Barigah et al. 2013).

Tous ces éléments plaident en faveur de l'implication de facteurs hydrauliques dans les phénomènes de dépérissements et de mortalité. Chez certaines espèces ayant une forte vulnérabilité à la cavitation, comme les peupliers (Hacke and Sauter 1995; Urli et al. 2013; Barigah et al. 2013), les dysfonctionnements du système hydraulique semblent même jouer un rôle majeur dans les événements de moralité liés des conditions de sécheresse (Zeppel et al. 2011; Anderegg et al. 2012). Toutes les études citées concernent en revanche des sécheresses à relativement court terme (pas plus de deux ou trois ans). De plus, la grande majorité des

travaux effectués concernent des sécheresses extrêmes. Les mécanismes liés aux dépérissements et à la mortalité en cas de sécheresse légère à modérée, mais chronique restent donc à éclaircir.

3.2.Hypothèse de limitation par le carbone

La régulation de la transpiration du houppier s'accompagne d'une diminution de l'assimilation photosynthétique de carbone (Weber and Gates 1990). Cela peut conduire l'arbre à utiliser ses réserves carbonées afin de subvenir à la demande métabolique (Bréda et al. 2006). En cas de sécheresse prolongée, l'arbre pourrait être amené à utiliser ses réserves carbonées jusqu'à leur épuisement (McDowell et al. 2008), ou jusqu'à ce que l'arbre soit dans l'impossibilité de les remobiliser (Sala et al. 2010). En climat tempéré, la gestion des réserves carbonées par l'arbre prend une importance particulière en hiver pour les espèces décidues. En effet, durant la période hivernale, les réserves sont la seule source de substrats carbonés disponible pour assurer l'entretien des tissus existants (Chapin et al. 1990). Comme pour l'hypothèse hydraulique, plusieurs éléments dans la littérature soutiennent l'hypothèse d'une implication de mécanismes liés à une perturbation du bilan de carbone de l'arbre dans les phénomènes de dépérissement ou de mortalité.

L'étude des flux de carbone et leur réponse à des sécheresses accompagnées ou non d'une augmentation artificielle de température permet d'aborder la question du rôle du bilan de carbone dans les événements de dépérissements et de mortalité. Ainsi, chez *Pinus edulis*, une augmentation de température conduit à une mortalité plus précoce lors d'une sécheresse (Adams et al. 2009a). Les auteurs de l'étude attribuent les différences de vitesse d'apparition de la mortalité à une augmentation des coûts respiratoires avec l'augmentation de la température, ce qui conduirait à un épuisement plus précoce des réserves carbonées (Adams et al. 2009a). Les réserves carbonées n'ont en revanche pas été mesurées directement, et la

fonction hydraulique des plants n'a pas été prise en compte dans cette étude. Sala (2009) et Leuzinger et al. (2009) soulignent que ces deux aspects limitent fortement l'interprétation des résultats. Malgré ces limites, une sécheresse peut induire un bilan de carbone négatif, comme montré par Zhao et al. (2013) sur des jeunes plants de Thuya. L'occurrence d'un bilan de carbone négatif dépend alors des conditions de température : une augmentation de température associée à la sécheresse conduit à court terme à un bilan de carbone négatif (Zhao et al. 2013).

Pour évaluer la possibilité d'un épuisement des réserves carbonées de l'arbre lors de sécheresses, il est également possible de suivre leurs concentrations en réponse à des conditions où l'eau est un facteur limitant (Leuzinger et al. 2009). Une littérature abondante s'est développée récemment sur le sujet (Tableau I.1). Suite à des sécheresses exceptionnelles, il a par exemple été montré que les arbres défoliés peuvent présenter des concentrations en réserves carbonées plus faibles que celles d'arbres dont les houppiers sont moins abimés, et ce juste après l'événement de sécheresse (Bréda et al. 2006), ou plusieurs années après (Galiano et al. 2011; Galiano et al. 2012). Les diminutions de concentrations en réserves consécutives à une sécheresse ne sont pas toujours homogènes dans l'ensemble des organes de l'arbre : elles peuvent ne concerner par exemple que les organes souterrains (racines et/ou lignotubers, Galiano et al. 2012; Hartmann et al. 2013b). Un épuisement complet des réserves carbonées suite à une sécheresse n'a en revanche jamais été documenté à ce jour.

En réponse à une sécheresse, une diminution des réserves carbonées n'est pas systématiquement observée. Certains auteurs documentent en effet une augmentation des réserves carbonées au moins dans certains organes en réponse à une sécheresse (Galvez et al. 2011; Anderegg 2012; Jacquet et al. 2014), tandis que dans d'autres cas, une sécheresse n'entraine pas de variation significative des réserves carbonées (Gruber et al. 2011; Gérard and Bréda 2012; Duan et al. 2013; Klein et al. 2014). Il semble que la réponse des réserves

carbonées dépende en partie des caractéristiques écophysiologiques de l'espèce étudiée. Par exemple, chez deux espèces du genre *Nothofagus* soumises à une sécheresse extrême, l'espèce la plus sensible à la sécheresse présente des concentrations en réserves carbonées diminuées, alors que l'espèce la plus résistante à la sécheresse présente des concentrations en réserves carbonées augmentées (Piper 2011). Les caractéristiques écophysiologiques d'une espèce ne sont cependant pas le seul déterminant de la réponse des réserves carbonées à un stress : on observe ainsi parfois pour une même espèce des évolutions de concentrations en réserves différentes à la suite d'une sécheresse (Tableau I.1).

Les réserves carbonées présentent donc une grande variété d'évolutions possibles en réponse à un stress, sans qu'il soit possible de dégager de grands déterminants de ces patterns (par exemple angiospermes vs. conifères, décidus vs. sempervirentes, isohydriques vs. anisohydriques). Pour tenter de comprendre cette diversité de réponses, il est nécessaire de prendre en compte que les fonctionnements carboné et hydraulique des arbres sont extrêmement interdépendants (McDowell 2011).

Espèce	Classe d'âge	Fonctionnement stomatique	Climat - Pays	Type de stress	Timing des mesures par rapport au stress	Mortalité ?	Organes étudiés	Composés mesurés	Réponse des reserves carbonées	Référence
Arbutus unedo	Arbres matures	Isohydrique	Méditerranéen – Espagne	Sécheresse expérimentale (exclusion de pluies)	Simultané	Non	Feuilles, branches, lignotubers	Sucres solubles, amidon	↘ (lignotubers uniquement)	(Rosas et al. 2013)
Eucalyptus globulus	Jeunes plants	Isohydrique	Sous serre - Australie	Sécheresse expérimentale	Simultanément	Non	Feuilles, tronc, racines	Sucres solubles, amidon	→	(Duan et al. 2013)
Fagus sylvatica	Arbres matures	Médian	Continental tempéré – France	Sécheresse naturelle	Immédiatement après	Non	Tronc	Sucres solubles, amidon	↘ (amidon uniquement)	(Bréda et al. 2006)
Fagus sylvatica	Arbres matures	Médian	Continental tempéré – France	Compaction des sols, mauvaise gestion sylvicole	4 ans après le début des symptômes	Non	Tronc	Sucres solubles, amidon	→	(Gérard and Bréda 2012)
Juniperus monosperma	Arbres matures	Anisohydrique	Semi-aride – USA (Nouveau Mexique)	Sécheresse expérimentale (exclusion de pluies)	Simultanément	Oui	Feuilles	Sucres solubles, amidon	↘	(Dickman et al. 2015)
Nothofagus dombeyi	Jeunes plants	Isohydrique	Tempéré maritime - Chili	Sécheresse expérimentale	Immédiatement après	Oui	Troncs, racines	Sucres solubles, amidon	↗	(Piper 2011)
Nothofagus nitida	Jeunes plants	Isohydrique	Tempéré maritime -Chili	Sécheresse expérimentale	Immédiatement après	Oui	Troncs, racines	Sucres solubles, amidon	↘	(Piper 2011)
Phillyrea latifolia	Arbres matures	Anisohydrique	Méditerranéen - Espagne	Sécheresse expérimentale (exclusion de pluies)	Simultané	Non	Feuilles, branches, lignotubers	Sucres solubles, amidon	→	(Rosas et al. 2013)
Picea abies	Jeunes plants	Isohydrique	Continental humide – Allemagne	Sécheresse expérimentale	Simultané	Non	Feuilles, branches, racines	Réserves C totales	↘ (dans les racines uniquement)	(Hartmann et al. 2013b)
Picea abies	Jeunes plants	Isohydrique	Continental humide – Allemagne	Sécheresse expérimentale et faible [CO_2]	Simultanément	Oui	Feuilles, branches, racines	Sucres solubles, amidon	↘ (faible [CO_2]) → (sécheresse)	(Hartmann et al. 2013a)
Pinus edulis	Jeunes arbres	Isohydrique	Semi-aride – USA (Nouveau Mexique)	Sécheresse expérimentale	Simultané	oui	Feuilles	Sucres solubles, amidon	↘	(Adams et al. 2013)
Pinus edulis	Arbres matures	Isohydrique	Semi-aride – USA (Nouveau Mexique)	Sécheresse expérimentale (exclusion de pluies)	Simultané	Oui	Feuilles	Sucres solubles, amidon	↘	(Dickman et al. 2015)
Pinus halepensis	Arbres matures	Isohydrique	Semi-aride – Israël	Sécheresse naturelle	3 à 4 ans après	Non	Branches, racines	Sucres solubles, amidon	→ (branches) ↘ (racines)	(Klein et al. 2014)

Espèce	Classe d'âge	Fonctionnement stomatique	Climat - Pays	Type de stress	Timing des mesures par rapport au stress	Mortalité ?	Organes étudiés	Composés mesurés	Réponse des reserves carbonées	Référence
Pinus pinaster	Jeunes arbres	Isohydrique	Thermo-atlantique - France	Sécheresse et défoliation expérimentales	Simultanément et immédiatement après	Non	Feuilles, tronc (xylème et phloème), racines	Réserves C totales	→ (arbre entier et racines) ↗ (xylème du tronc)	(Jacquet et al. 2014)
Pinus sylvestris	Arbres matures	Isohydrique	Tempéré océanique - Espagne	Sécheresse naturelle	4 ans après	Non	Tronc (aubier)	Réserves C totales	↘	(Galiano et al. 2011)
Pinus sylvestris	Arbres matures	Isohydrique	Continental tempéré - Autriche	Sécheresse naturelle	Simultané	Non	Feuilles, branches, tronc (aubier), grosses racines	Sucres solubles, amidon	→	(Gruber et al. 2011)
Populus balsamifera	Jeunes plants	Isohydrique	Continental sec – Canada (Alberta)	Sécheresse	Simultanément	Oui	Feuilles, tronc, racines	Sucres solubles, amidon	→ (sucres solubles) ↘ (amidon)	(Galvez et al. 2013)
Populus tremuloides	Jeunes arbres	Isohydrique	Semi-aride – USA (Colorado)	Sécheresse expérimentale	Simultané et immédiatement après	Non	Feuilles, branches, racines	Sucres solubles, amidon	↗	(Anderegg 2012)
Populus tremuloides	Jeunes plants	Isohydrique	Continental sec – Alberta (Canada)	Sécheresse expérimentale	Simultané (3 mois)	Non	Feuilles, racines	Sucres soluble, amidon	↗	(Galvez et al. 2011)
Populus tremuloides	Jeunes plants	Isohydrique	Continental sec – Canada (Alberta)	Sécheresse	Simultanément	Oui	Feuilles, tronc, racines	Sucres solubles, amidon	→ (sucres solubles) ↘ (amidon)	(Galvez et al. 2013)
Pseudotsuga menziessi	Arbres matures	Isohydrique	Pacifique maritime – USA (Oregon)	Défoliation naturelle	Simultanément	Non	Feuilles, branches, tronc	Sucres solubles, amidon	→ (branches et feuilles) ↘ (tronc)	(Saffell et al. 2014)
Quercus ilex	Arbres matures	Isohydrique	Tempéré océanique - Espagne	Sécheresse naturelle	7 ans après	Non	Lignotubers	Réserves C totales	↘	(Galiano et al. 2012)
Quercus ilex	Arbres matures	Isohydrique	Méditerranéen - Espagne	Sécheresse expérimentale (exclusion de pluies)	Simultané	Non	Feuilles, branches, lignotubers	Sucres solubles, amidon	→	(Rosas et al. 2013)
Quercus petraea	Arbres matures	Médian	Continental tempéré - France	Sécheresse naturelle	Immédiatement après	Non	Tronc	Amidon	↘	(Bréda et al. 2006)
Quercus velutina	Jeunes plants	Anisohydrique	Continental humide – USA (New Jersey)	Défoliation expérimentale	Immédiatement après et 5 mois après	Non	Feuilles, tronc, racines	Amidon	↘ immédiatement après → ou ↗ 5 mois après (selon traitements)	(Wiley et al. 2013)

Tableau I.1 Bilan bibliographique de la réponse des réserves carbonées suite à différents stress. Le fonctionnement stomatique indique de quel coté du continuum isohydrique-anisohydrique se trouve l'espèce, et non un comportement strictement iso ou anisohydrique. Il est également précisé si le stress entraine ou non la mort des arbres.

3.3.Liens fonctionnels entre les fonctionnements hydrauliques et carbonés

Un premier niveau de couplage des fonctionnements hydraulique et carboné se situe au niveau de la régulation stomatique des échanges gazeux. En effet, les échanges gazeux ne peuvent avoir lieu que pour une certaine gamme de potentiels hydriques foliaires (Sperry 2000). Le potentiel hydrique foliaire est lui-même entre autres lié au fonctionnement hydraulique de l'arbre (Sperry 2000). Au niveau des feuilles, les fonctionnements hydraulique et carboné sont donc indissociables. Lors de sécheresses, la régulation des échanges gazeux nécessite donc de trouver un compromis entre assimilation de carbone et contrôle de la tension de la colonne d'eau dans le xylème (Bréda et al. 2006). Il apparait que la consommation des réserves carbonées lors d'une sécheresse dépend en partie de processus relevant du fonctionnement hydraulique de l'arbre (McDowell et al. 2008). Chez deux espèces de conifères ayant des vulnérabilités à la cavitation contrastées, il a ainsi été montré que lors d'une sécheresse extrême, la mortalité était liée à une limitation hydraulique des échanges gazeux, et non à la défaillance complète du système hydraulique (Plaut et al. 2012). Ces limites hydrauliques conduisent à une acclimatation des échanges gazeux à des conditions de sécheresses (Limousin et al. 2013). Il est alors observé un compromis entre capacité photosynthétique maximale et résistance de la photosynthèse à la sécheresse (Limousin et al. 2013).

Une fois assimilé par les feuilles, le carbone est transporté vers des organes puits, où il sera utilisé ou stocké. Les organes de stockage deviennent par la suite des sources de carbone. Or, certaines fonctions de puits sont directement dépendantes de l'état hydrique de l'arbre. C'est par exemple le cas de la croissance radiale du tronc, la pression de turgescence conditionnant les processus d'expansion cellulaire (Zweifel et al. 2006). Une diminution de la demande en carbone des puits liée à une limitation hydrique de ces derniers peut en conséquence engendrer une augmentation des réserves carbonées pendant au moins une partie

de la sécheresse (Piper 2011). Par ailleurs, la demande en substrat carboné est parfois spatialement éloignée des organes de production ou de stockage (Dickson 1989). Le transport de sucres dans le phloème est alors crucial pour assurer l'approvisionnement en sucres des organes puits (Sala et al. 2010). Une défaillance du transport phloémien suite à un faible approvisionnement en eau et/ou en photoassimilats lors d'une sécheresse pourrait conduire à un isolement des organes sources des organes puits, engendrant des déplétions locales en réserves (Sala et al. 2010; McDowell 2011). Alternativement, une défaillance du transport phloémien pourrait engendrer un dysfonctionnement hydraulique du xylème en empêchant l'apport de sucres nécessaire à la régulation osmotique de la sève brute, voire au refilling des vaisseaux embolisés (McDowell 2011; Sevanto et al. 2014).

3.4. Hypothèses complémentaires : fonctionnement azoté et transfert de carbone au sol

Les deux hypothèses de mort par défaillance du système hydraulique ou par déficience en réserves carbonées ne sont donc pas mutuellement exclusives, les deux mécanismes pouvant entrer en interaction. Il est donc indispensable de prendre ces deux aspects en compte lors d'études visant à expliciter les mécanismes fonctionnels sous-jacents à la réponse des arbres à des stress. En revanche, d'autres aspects des réponses écophysiologiques des arbres lors de dépérissements restent très mal connus.

3.4.1. Dynamique interne de l'azote

C'est le cas de la dynamique interne de l'azote dans l'arbre. En effet, aucune étude à ce jour n'a évalué la réponse des concentrations et des dynamiques de réserves azotées lors d'un dépérissement. Or, les réserves azotées jouent un rôle crucial, notamment pour la reprise de la croissance au printemps (Wildhagen et al. 2010; El Zein et al. 2011b; Bazot et al. 2013). En

effet, lors de la formation des branches et des nouvelles feuilles, l'absorption racinaire de l'azote du sol est dans un premier temps très faible, et la croissance des nouveaux organes dépend pendant cette période quasiment exclusivement de la remobilisation de l'azote stocké dans les organes pérennes (Geßler et al. 1998; El Zein et al. 2011a; Bazot et al 2015 in prep). Dans un second temps, deux à trois semaines après le débourrement, l'absorption racinaire de l'azote du sol prend le relai sur les réserves azotées pour la formation des nouveaux organes (Geßler et al. 1998; El Zein et al. 2011a; Bazot et al 2015 in prep). Par ailleurs, à l'automne, concomitamment à la remobilisation de l'azote foliaire pour la formation des réserves, on observe un deuxième pic d'absorption racinaire d'azote du sol (Geßler et al. 1998; Bazot et al 2015 in prep). Le remplissage automnal des réserves azotées à dépend donc à la fois de facteurs internes à l'arbre et de facteurs liés au sol.

Au cours d'un dépérissement, la surface foliaire totale de l'arbre et la biomasse des branches fines sont fortement réduites. Ces organes sont riches en azote, et jouent un rôle important dans la dynamique interne de l'azote dans l'arbre (Bazot et al. 2013). La dynamique interne de l'azote pourrait par conséquent être perturbée par l'augmentation de la mortalité des branches fines lors d'un dépérissement. De manière plus générale, l'effet de stress sur le fonctionnement azoté des arbres reste assez mal connu. Lors d'expériences de stress hydrique, il a été montré que l'absorption d'azote pouvait se trouver réduite (Fotelli et al. 2002; Geßler et al. 2004). Une diminution de l'absorption d'azote n'est cependant pas toujours observée lors de sécheresses (Fotelli et al. 2004; Peuke and Rennenberg 2004). Les concentrations en réserves azotées sont en partie déterminées par l'absorption d'azote du sol par les racines (Millard and Grelet 2010). Une modification de l'absorption d'azote par les racines pourrait donc également impacter les concentrations et/ou les dynamiques saisonnières réserves azotées de l'arbre.

3.4.2. Transfert de carbone vers le sol

Les racines des arbres assurent l'absorption d'eau et des nutriments du sol. Les racines des arbres modifient les propriétés du sol (capacité de rétention d'eau, pH…) dans leur environnement immédiat, en relâchant des composés carbonés, dans un processus appelé rhizodéposition (Lynch and Whipps 1990). La rhizodéposition fournit des substrats carbonés aux communautés microbiennes du sol (Lynch and Whipps 1990). L'activité biologique dans la rhizosphère, stimulée par les rhizodépots, est plus forte dans le sol rhizosphérique que dans le sol non rhizosphérique (Lynch and Whipps 1990). En retour, les communautés microbiennes de la rhizosphère peuvent avoir des effets positifs sur la productivité des plantes en modifiant par exemple la disponibilité en certains nutriments, notamment l'azote (Van Der Heijden et al. 2008). Les microorganismes de la rhizosphère peuvent également impacter les plantes de façon négative, soit directement en agissant en tant que pathogènes, soit indirectement en entrant en compétition avec les plantes pour l'absorption des nutriments (Van Der Heijden et al. 2008).

Les interactions entre les plantes et les communautés microbiennes du sol via la rhizodéposition ont donc une très grande importance à la fois par leurs effets sur la disponibilité en eau et en nutriments dans l'environnement immédiat des racines, mais aussi à cause de l'importance quantitative de la rhizodéposition : on estime en effet que chez les arbres, une part importante du carbone assimilé serait transféré à la rhizosphère (Grayston et al. 1997). Cette importance est telle que dans le cadre d'une approche intégrée du fonctionnement de l'arbre, le flux de carbone vers le sol peut être considéré comme un puits de carbone à part entière, au même titre que d' autres grandes fonctions de l'arbre comme par exemple la croissance ou la respiration (Högberg and Read 2006).

A ce jour, aucune étude ne s'est penchée sur l'effet d'un dépérissement sur la rhizodéposition. En revanche, des expériences de girdling ont montré qu'une diminution du

transfert de carbone vers le sol avait des conséquences négatives sur les communautés microbiennes des sols, à la fois en terme d'abondance (Högberg and Högberg 2002) et de diversité (Schulze et al. 2005; Koranda et al. 2011). On peut par conséquent penser qu'un dépérissement, lors duquel l'assimilation de carbone est réduite du fait de la diminution de la surface foliaire totale (Anderegg et al. 2014), pourrait modifier la quantité et/ ou la qualité des rhizodépots et par conséquent les communautés microbiennes du sol. Une première étude sur un cas inexpliqué de dépérissement chez l'Eucalyptus a ainsi montré des différences d'activités cataboliques dans les communautés microbiennes de la rhizosphère d'arbres poussant dans des parcelles saines et dépérissantes (Cai et al. 2010). Plus précisément, une capacité augmentée des communautés bactériennes du sol à dégrader certains acides organiques pourrait conduire sur les sites dépérissants à une plus faible disponibilité en nutriments minéraux dans le sol par rapport aux sites sains, en particulier pendant la saison sèche (Cai et al. 2010).

En raison des nombreuses interactions entre les fonctionnements hydraulique, carboné et azoté, la réponse d'une plante à un ou des stress doit être abordée d'une manière la plus intégrative possible, afin de prendre en compte au mieux les grandes fonctions de l'arbre et leurs interactions. Dans ce travail, c'est ce type d'approche qui est utilisé pour essayer de comprendre les mécanismes fonctionnels sous- jacents à un dépérissement de hêtres en forêt de Fontainebleau.

4. Objectifs de la thèse

Comme vu précédemment, les dépérissements forestiers sont des phénomènes complexes, dont les mécanismes fonctionnels sous-jacents restent mal cernés. C'est particulièrement vrai pour les dépérissements se déroulant sur le long terme, la majorité des études concernant uniquement les réponses à court terme des plantes aux stress (Tableau I.1). L'objectif principal de cette thèse est de documenter les mécanismes écophysiologiques à l'œuvre dans un dépérissement de hêtres matures, lié au moins en partie à des sécheresses répétées. Ce dépérissement se déroule sur le long terme, comme indiqué par les observations de terrain et une étude dendrochronologique (Silva 2010, Figure I.4). Il sera examiné ici en dehors de fortes contraintes climatiques.

L'originalité de ce travail repose dans l'approche intégrative adoptée à l'échelle du système arbre-sol. Sur un même groupe d'arbres, nous avons en effet étudié des caractéristiques liées aux fonctionnements hydraulique, carboné et azoté des arbres. De plus, les interactions entre les arbres et les communautés microbiennes des sols, potentiellement importantes pour l'absorption de l'eau et la nutrition minérale, ont également été abordées. Les aspects azote et sol sont particulièrement novateurs, et très peu documentés dans la littérature sur les dépérissements forestiers. Ce travail sera structuré en trois parties, chacune des parties étant constituée d'un article, soumis ou en préparation.

Chapitre 1 : fonctionnements hydraulique, carboné et azoté des arbres sains et dépérissants pendant la saison de végétation.

Lors de dépérissements, les études prenant en compte concomitamment les fonctionnements hydraulique et carboné d'arbres matures sont peu fréquentes. De plus, les réponses des concentrations et des dynamiques des réserves azotées lors de dépérissements n'ont jamais été documentées, en dépit de leur importance notamment pour la croissance

printanière (Wildhagen et al. 2010; Bazot et al. 2013). On peut donc se demander si les fonctionnements hydraulique, carboné et azoté des arbres dépérissants sont modifiés pendant la saison de végétation. Dans ce chapitre, nous analysons des caractéristiques des fonctionnements hydraulique, carboné et azoté d'arbres sains et dépérissants pendant la saison de végétation. Sur deux ans, la croissance radiale du tronc, ainsi que les concentrations minimales et maximales en réserves carbonées et azotées sont documentées. Durant la deuxième année de l'étude, le fonctionnement hydraulique des arbres est caractérisé, et le fonctionnement foliaire est examiné via l'utilisation de proxys. Cet article a été soumis à « Trees – structure and function ».

Chapitre 2 : fonctionnement hivernal des arbres sains et dépérissants.

Après avoir caractérisé le fonctionnement estival des arbres sains et dépérissants, nous abordons une thématique assez peu documentée dans la littérature : le fonctionnement hivernal des arbres. En climat tempéré, les arbres décidus dépendent en effet de leurs réserves (en particulier carbonées) pour assurer l'entretien des tissus vivants ainsi que la résistance au gel. Chez des arbres sains et dépérissants, nous avons mesuré la respiration d'entretien des troncs en hiver, ainsi que la température engendrant 50% de mortalité des cellules vivantes du xylème. En parallèle, les concentrations en réserves carbonées et azotées ont été documentées. Le stockage et la remobilisation des réserves carbonées et azotées dans le xylème reposent sur les cellules de parenchyme (Tromp 1983). Ils seront ainsi mis en relation avec des caractéristiques du bois, notamment la quantité et la structure des rayons ligneux. L'article présenté est en préparation et sera prochainement soumis.

Chapitre 3 : flux de carbone vers le sol et les racines chez des arbres sains et dépérissants.

Dans les deux chapitres précédents, nous nous sommes essentiellement intéressés aux compartiments aériens de l'arbre. Les processus liés au compartiment souterrain (considéré ici comme l'ensemble formé par les racines et le sol rhizosphérique) lors de dépérissements forestiers restent en revanche très mal documentés, en dépit de leur importance potentielle notamment pour la disponibilité en eau et en nutriment dans l'environnement immédiat des racines. La question suivante est donc posée : le dépérissement s'accompagne-t-il de caractéristiques différentes des racines et/ou des communautés bactériennes de la rhizosphère ? Dans ce chapitre, les densités et croissances racinaires des arbres sains et dépérissants sont comparées. La quantité et la qualité des rhizodépots sont estimées via l'utilisation de proxys. L'article présenté est en préparation. Il sera complété par des résultats sur la structure des communautés microbiennes issus de techniques de biologie moléculaire, dont l'acquisition est actuellement en cours.

II. Reduced stem growth, but no reserve depletion or hydraulic impairment in beech suffering from long-term decline

Reduced stem growth, but no reserve depletion or hydraulic impairment in beech suffering from long-term decline

ALICE DELAPORTE [123], STEPHANE BAZOT [123*], CLAIRE DAMESIN [123]

1. Université Paris-Sud, UMR 8079, Laboratoire Ecologie Systématique et Evolution, Orsay, F-91405, Orsay, France

2. CNRS, UMR 8079, Laboratoire Ecologie Systématique et Evolution, Orsay, F-91405, Orsay, France

3. AgroParisTech, UMR 8079, Laboratoire Ecologie Systématique et Evolution, F-75231 Paris, France

***Author for correspondence**

Stéphane Bazot

tel: (+33) 1 69 15 71 36

fax: (+33) 1 69 15 72 38

email: stephane.bazot@u-psud.fr

Key message Under non-extreme drought conditions, reduced stem growth is not associated with reserve or hydraulic impairment in beech suffering from long term decline.

Abstract

Global change is expected to increase the frequency and the intensity of drought events in temperate ecosystems. In some regions, this might be associated with an increase in tree decline. Of the ecophysiological mechanisms that have been proposed to explain tree decline, an impairment of the vascular transport system and/or carbon function are two of the most discussed. In a context of long term decline caused by droughts, we investigated the functional differences between the carbon, nitrogen, and hydraulic functions of healthy and declining mature beech (*Fagus sylvatica* L.) trees. The study was carried out over two consecutive years with contrasting water availabilities. The radial growth of declining trees was clearly less than that of healthy trees, due to a lower growth rate, associated during the wet year with a shorter growth period. Leaf functional characteristics and hydraulic parameters (native embolism and cavitation vulnerability curves) were similar in healthy and declining trees. However, at the end of a growing season characterized by a dry spring, carbon reserves concentrations in young branches of declining trees were lower than those in healthy trees, though they recovered during the following non-constraining growing season. Our results did not indicate carbon starvation, nitrogen deficiency, or hydraulic failure. However, there seems to be some compensation mechanism related to reserve dynamics in the remaining living tissue of the declining trees. This study shows that the climate conditions of successive years are probably crucial for these functional adjustments to be operational.

Key-words beech, reserves, forest decline, growth, hydraulic failure

Introduction

Climate change is resulting in more frequent and more intense droughts, and this tendency is expected to continue in the coming decades, particularly in temperate climates (Intergovernmental Panel on Climate Change 2014). At the same time, the frequency of drought-induced forest declines is increasing (Lorenz and Becher 2012). As a consequence, there has been renewed interest in studying forest decline in the scientific community (McDowell et al. 2008, Adams et al. 2009, Breshears et al. 2009, Leuzinger et al. 2009, Sala 2009, McDowell and Sevanto 2010). However, an accurate prediction of the amplitude of this increased tree mortality in coming decades is not possible (Allen et al. 2010), due to a lack of understanding of the functional mechanisms involved in the responses of trees to climate change. The functional mechanisms underlying tree decline therefore need to be investigated thoroughly (Bréda and Badeau 2008).

The potential roles of carbon (C) and/or water balances in tree decline or mortality during stressful events have long been suggested (Bossel 1986; Martínez-Vilalta et al. 2002). In a review based on field observations, Waring (1987) suggested that the C balance of trees is key to understand their reactions to diverse stressors. More recently, research efforts have focused on drought-induced tree mortality (McDowell et al. 2008; Anderegg et al. 2012; Adams et al. 2013; Hartmann et al. 2013b; Hartmann et al. 2013a). Two functional mechanisms underlying drought-induced tree mortality have been hypothesized: a failure of the hydraulic system; and carbon starvation. The latter is defined as either an exhaustion of C reserves (referred to as NSC - Non-Structural Carbohydrates) of the tree, or a failure to remobilize them (McDowell et al. 2008; Sala et al. 2010). The exclusivity of these two hypotheses has since been reconsidered (Sala et al. 2010; Sevanto et al. 2014). Moreover, it is now known that insects and/or pathogens can play a major role in tree decline (Durand-Gillmann et al. 2012).

Recent studies have investigated the roles of C and/or water balances during drought-related tree declines and mortality (Brodribb and Cochard 2009, Galiano et al. 2011, Galvez et al. 2011, Amoroso et al. 2012, Anderegg et al. 2012). Several studies of the role of C starvation have yielded contradictory results (Piper 2011; Hartmann et al. 2013b; Zhao et al. 2013). C starvation has been difficult to demonstrate empirically for three reasons: firstly, because this hypothesis relies on the assumption that C reserves accumulate mainly passively (Sala et al. 2010), which has recently been challenged (Wiley et al. 2013; Dietze et al. 2014); secondly, because the NSC pools are never fully depleted (Sala et al. 2010), suggesting that there is a threshold below which the NSC pools are too low to sustain the functioning of the tree in the absence of C assimilation (McDowell et al. 2011); and thirdly, because even if this threshold could be defined for each species, the tree might not be able to remobilize and/or transport the stored NSC (Sala et al. 2010).

Non-structural nitrogen (N) compounds supply N for spring growth and winter maintenance (Wildhagen et al. 2010; Bazot et al. 2013). However the tree internal N cycle has been largely overlooked in studies of drought-related tree decline, and to our knowledge, the N reserves of trees have seldom, if ever, been assessed during tree decline. N reserves are essential for long-term tree survival and health status, as N is often a limiting element in forest ecosystems (Waring 1987; LeBauer and Treseder 2008). As a consequence, we hypothesized that N reserves (here referred to as NSNC – Non-Structural Nitrogen Compounds) would be lower in declining trees than in healthy trees.

Studies of hydraulic failure, on the other hand, have shown that loss of hydraulic conductance via cavitation may be linked to tree mortality. Indeed, Urli et al. (2013) showed that in temperate angiosperms, a high degree of embolism (around 90%) was associated with tree mortality, and that drought resistance and cavitation resistance were strongly linked. Moreover, the link between the hydraulic limits of trees and drought-induced tree mortality

has been well documented, at both local and global scales (Brodribb and Cochard 2009; Poot and Veneklaas 2012; Choat et al. 2012; Nardini et al. 2013; Barigah et al. 2013). Several studies have also shown that hydraulic failure was the final mechanism causing death of drought-affected trees (Anderegg et al. 2012; Adams et al. 2013; Hartmann et al. 2013a). However, as pointed out by Plaut et al. (2012), hydraulic failure and C starvation might often interact when drought-related tree mortality occurs.

The ecophysiological differences between healthy and declining trees during long-term declines have seldom been studied. During these events, affected trees may survive for years, or even decades with severely thinned crowns. As described above, short-term responses of C and N reserve concentrations and hydraulic conductance to drought remain unclear. Understanding long term tree functional responses to repeated droughts is particularly critical for forest survival and predictions of the response of forest productivity to future climate scenarios (Bréda et al. 2006). Since reserve depletion may already be important after a severe drought (Bréda et al. 2006), it can be hypothesized that recurrent droughts would induce drastic decreases in reserves, which could lead to a progressive loss of tree resilience, by reducing the ability to regenerate (López et al. 2009).

This paper investigates the long-term ecophysiological responses of mature beech trees (*Fagus sylvatica* L.) to recurrent droughts (especially in 1959, 1976, 1990, 1992, 1996 and 2003). The study plot is an even-aged beech stand (mean tree age of 95 years) growing in the Fontainebleau state forest (France). Low soil water availability has been shown to be a predisposing factor of decline for beech in this forest, and a dendrochronological approach revealed the triggering role of dry years (Silva 2010). In the plot, two categories of trees can be distinguished in terms of their crown conditions: healthy trees with dense crowns; and declining trees with highly thinned crowns. Silva (2010) showed that until 1959, the growth of currently declining and healthy trees was similar, and that after the dry 1959 growing

season, radial growth of currently declining trees had been reduced. Since the 1996 drought, the currently declining trees displayed less interannual growth variation than the currently healthy trees. Furthermore, crown loss in the declining trees also started at the end of the 1990s (Silva 2010), and no biotic attack has been recorded since the regeneration of this stand in the 1910s (National Forest Office, personal communication). Microsite soil water availability is not linked to the health status of the trees (Silva 2010).

In this study, we intended to determine whether the altered growth of declining trees was associated with reduced internal carbon and/or nitrogen reserve concentrations, and/or with hydraulic dysfunction. More precisely, we asked the following questions:

i. To what extent and how is the stem radial growth reduced in declining trees at the seasonal time scale? Does it depend on climatic conditions?
ii. Is the growth reduction associated with altered foliar functioning?
iii. Is the altered seasonal growth in declining trees related to a dysfunction in reserve mobilization and/or storage?
iv. Is water transport damaged during the growing season in declining trees?

To answer these questions, tree radial growth was monitored weekly over two growing seasons with contrasting climatic conditions: one with a spring drought (2011); and one without water constraint (2012). Together with stem radial growth, the seasonal changes of C and N reserve concentrations were assessed over two years in stems and young branches. In 2012, foliar functioning was characterized by the percentage of N in the total organic matter, carbon isotope composition ($\delta^{13}C$) and stomatal density measurements on mature leaves. Hydraulic functioning was studied at the branch level by measurements of water potential, vulnerability to cavitation and native embolism.

Materials and methods

Site and stand description

The study was carried out in the Fontainebleau state forest, France (48°22' N, 02°36' E, mean elevation 120 m a.s.l.) during the 2011 and 2012 growing seasons. This forest extends over 17,000 ha, 60 km southeast of Paris. The climate is temperate, with a mean annual temperature of 10.6°C and mean annual precipitation of 749 mm, well distributed throughout the year (for the 1960-2010 period). During the 1960-2010 growing seasons (from March to September), mean temperature was 14.1°C, mean precipitation was 426 mm, and there were on average 91 days with precipitation. The 2011 growing season was remarkable for being unusually dry and warm, particularly in spring: the mean temperature was 15.1°C, precipitation was 307 mm and the number of days with precipitation was 69. In contrast, the 2012 growing season had average climatic conditions, with a mean temperature of 14.4°C, precipitation of 364 mm and 95 days with precipitation.

The study site is a mature, monospecific beech (*Fagus sylvatica* L.) stand which has shown strong signs of decline since the late 1990s (observation by the forester in charge of the plot), where mean tree age in 2011 was 95 years and dominant height was 27 m. During the decline, the thinning of the crown has been observed to be episodic. The forester in charge of the plot has observed that crown loss episodes usually coincided with low precipitation periods. The crown condition typically does not improve during favorable years (with abundant and well temporally-distributed precipitation). Declining trees typically die after ca. 20 years. During the 2011 growing season, death of upper branches was observed in the crowns of declining trees, resulting in further thinning of their crowns. Despite more favorable climatic conditions in 2012, the crown health of declining trees did not improve.

Trees were growing on a rather shallow podzoluvisol soil (mean profile depth 0.6 m), with a C:N ratio of 18.7:1, and an extractable soil water content of 101 mm. The soil texture is dominated by Stampian sand mixed with loam and clay at different depths.

Sampling design

From the dominant storey, five trees with altered crowns ("declining") and five trees with intact crowns ("healthy") were chosen for the present survey. The crown loss was evaluated using the DEPEFEU (DEPErissement des FEUillus) protocol (Nageleisen and Goudet 2011), which quantifies crown thinning on a scale from 0 (healthy tree) to 4 (dead tree). The selected healthy trees had large, dense crowns and abundant fine ramification (average DEPEFEU score: 0.9), while the declining trees had reduced crown areas, very transparent crowns, only one or two main branches remaining and very limited fine ramification (average DEPEFEU score: 3.6). On average, declining trees had 70% less leaf area than healthy trees, as estimated with the DEPEFEU protocol. The mean diameters at breast height (45.9 ± 5 cm) and the heights of the two groups (26.5 ± 4 m) were similar. Radial stem growth was followed weekly with manual band dendrometers (DB20, EMS Brno, Brno, Czech Republic) placed at breast height. The key phenological stages (budburst, evolution of the Leaf Mass per Area –LMA and yellowing) were also monitored.

Sampling for C and N measurements

Trees were sampled twice in both 2011 and 2012: at budburst (50% of the tree buds broken); and at yellowing (senescence of 50% of tree leaves). The time of budburst was determined as described in Vitasse et al. (2009): a bud was considered broken when its leaves started unfolding. In beech, budburst has been shown to correspond to minimum reserve

concentrations, while at yellowing the reserve concentrations are at a maximum (Barbaroux and Bréda 2002; Hoch et al. 2003).

On each sampling date, we collected: light-exposed branches formed in 2010 using a shotgun; two trunk cores taken at breast height (one for C reserve assessment, one for N reserve assessment) containing phloem and xylem; and two coarse root cores (diameter ≥ 5 mm, see Le Goff and Ottorini 2001, taken approximately 50 cm from the trunk). Sampling was always performed between 10:00 and 12:00.

Samples were transported in a cooler to the laboratory, where their fresh weight was determined. The samples were then immediately placed in a freezer at -80°C. The samples destined for carbohydrate analysis were lyophilized for 7 days, and dry weight determined immediately after freeze-drying to estimate the humidity content of each organ. The samples destined for soluble protein assessment were kept frozen until analysis.

Light-exposed leaves were collected during the 2012 growing season, lyophilized and ground to a fine powder. 1 mg subsamples were then placed in tin capsules in order to determine the $\delta^{13}C$ and percentage of N with a CN analyzer and a mass spectrometer (Sercon Ltd., Cheshire, UK).

Hydraulic parameters

Water potentials

Pre-dawn potentials were measured twice in each tree during the 2011 and 2012 growing season (on 2011/06/29, 2011/08/23, 2012/05/25 and 2012/08/20) in three light-exposed branches, between 10 and 20 cm long. Midday water potentials were measured only in 2012 growing season. Measurements were immediately made in the field using a pressure chamber (Model 600, PMS Instruments, USA), after recutting 2 cm away from the last visible lead shot injury.

Native embolism

Light-exposed branches in the upper third part of the crown of each tree were sampled three times during the 2012 growing season (on 2012/04/25, 2012/05/29 and 2012/09/07) with a shotgun. The whole branch was immediately wrapped in moist paper towels, placed in a black plastic bag (to prevent any photosynthesis and transpiration) and brought back to the laboratory in a cooler. They were then preserved in a cold room at 7°C until analysis. Native embolism (% loss of hydraulic conductivity, PLC) on ca. 3 cm branch segments using a XYL'EM® embolism meter (Bronkhorst, Montigny-les-Cormeilles, France). The technique requires hydraulic conductivity measurements before and after water refilling (for further details, see Cochard 2002). The branch segments were allowed to relax for ca. 60 minutes before measurement to prevent any measurement artifact (Wheeler et al. 2013), then perfused with a degassed 100 mmol l^{-1} KCl – 10 mmol l^{-1} $CaCl_2$ solution, filtered at 0.2 µm. Samples were flushed 3 times for 3 seconds at 0.2 MPa to remove embolism. Native embolism was computed as described in Sperry et al. (1988).

Cavitation vulnerability curves

To establish vulnerability curves, two south-oriented light-exposed branches from each tree were sampled at the end of May 2012. From each branch, segments ca. 20 cm long were selected. The average age of these segments was 20 years. Vulnerability curves were established using the Cavitron technique (Cochard 2002), as described in Wortemann et al. (2011). Beech vessel length (90% of beech vessels are shorter than 12 cm, see Hacke and Sauter 1995) is ideal to establish reliable vulnerability curves, as discussed in Cochard et al. (2010). The slope (% MPa^{-1}), xylem pressure inducing losses of conductance of 12% (P_{12}), 50% (P_{50}) and 88% (P_{88}) were computed as described in Wortemann et al. (2011). P_{12} is considered as the xylem pressure at which embolism begins to appear in the xylem conduits

(Sparks and Black 1999), and P_{88} the xylem pressure at which the embolism level in the xylem conduits becomes critical (Domec and Gartner 2001).

Foliar functioning characterization

During the 2012 growing season, light-exposed leaves were sampled at the same phenological stages as trees were sampled for C and N measurements. The total area of 10 leaves randomly selected from each tree was measured using an area-meter (Delta-T Area-meter, Delta-T Devices, UK). These 10 leaves were then dried at 60°C for four days, and their LMA computed.

In mid-August 2012, 10 sun-exposed leaves were sampled in the upper third of the crown. On each of these leaves, a 1 cm² area was covered with a thin layer of nail polish, and left to dry for approximately 20 min. This was then carefully removed with a piece of clear adhesive tape, and mounted on a glass slide. With a magnification of 200 times under an optical microscope (Leitz Wetzlar, Germany), stomata were counted in three separate fields from each leaf. The field width was 930 µm, as calculated with a graduated slide. The stomatal densities of the three fields were averaged to obtain the mean stomatal density of each leaf (number of stomata mm^{-2}). The average stomatal densities of each of the 10 leaves of a given tree were then averaged to obtain the tree's average stomatal density.

Quantification of N and C compounds

Total non-structural N (TNSN)

Soluble proteins were assessed in the 2010 growth units, the phloem, the outermost 1.5 cm of xylem and the coarse roots, using an adaptation of the protocol described by Bahrman et al. (1997). 400 mg of fresh matter were ground in liquid N. The proteins were then precipitated with 10 ml of precipitation solution (acetone, 10% TCA and 0.07% β-

mercapto-ethanol). After a night at -20°C, the extracts were centrifuged (15 min, -4°C, 12000 *g*), and the supernatants eliminated. The remaining pellets were rinsed with 10 ml 0.07% β-mercapto-ethanol in acetone, incubated for 1 hour at -20°C, centrifuged (30 min, 4°C, 12000 *g*), and the supernatants were again eliminated. The pellets were then dried in a centrifugal vacuum concentrator (Centrivap, Labconco, USA), and 10 - 20 µl per mg of pellet of a solubilization solution (7M urea, 2M thiourea, 100mM DTT, 4% CHAPS, 0.4% Triton x100 in water) were added. After sonication during 30 min, the tubes were centrifuged (30 min, 15°C, 12000 *g*), and the supernatants were transferred to other tubes.

Proteins were assessed in these supernatants by the Bradford method (Bradford 1976). Bradford reagent (BioRad Protein Assay, BioRad, USA) was diluted four times, and 3.5 ml of the diluted dye reagent were added to 10 µl of the protein extract mixed with 10 µl of HCl 0.1 mol l^{-1} and 80 µl of distilled water. After 15 min in the dark, absorbance was read at 595 nm with a spectrophotometer (UVIKON 938, NorthStar, UK). Protein concentration was calculated using solutions of increasing ovalbumine concentrations as standards. The protein concentrations were expressed in grams of reserve compound per 100 grams of dry matter (g 100 g DW^{-1}).

Non structural carbohydrates

Soluble sugars and starch were extracted from 10 mg (± 0.5 mg) of finely ground dry matter from the 2010 growth units, phloem, outermost 0.5 cm of xylem and coarse roots. Samples were ground using a ball mill (MM 301, Retsch, Germany) with 5 mm balls shaken at 30 Hz three times for 2 min. Soluble sugars were extracted twice with 1 ml of boiling 80% ethanol. These 2 ml were pooled and centrifuged. The supernatants and the pellets containing starch were dried in a centrifugal vacuum concentrator to remove ethanol (Centrivap, Labconco, USA). Soluble sugars and the pellets containing starch were then rehydrated with

0.02 mol l^{-1} NaOH. Glucose, fructose and sucrose were assessed enzymatically by the method described in Barbaroux et al. (2003) and Brummer and Cui (2005). Their concentrations were then added to determine the concentration of total soluble sugars. Starch was quantified as the glucose equivalent, using the enzymatic method described for soluble sugars. The concentrations of total non-structural carbohydrates (TNSC), starch and total soluble sugars were expressed in grams of reserve compound per 100 grams of dry matter (g 100 g DW^{-1}).

Statistical analyses

The growth data of each tree for each year were fitted with a sigmoid curve using Matlab software (MATLAB 2008a, MathWorks, Natick, Massachusetts, USA):

$$CG = \frac{a}{1 + e^{-S*(D-b)}}$$

in which CG is the cumulative stem diameter growth (mm), *a* is the upper asymptote (mm), *S* is the growth rate (mm day^{-1}), *D* is the date (day of year) and *b* is the inflexion point of the curve.

The dates of start and end of growth were determined by deriving the function six times for the start date and five times for the end date. The dates obtained with the fitted curves were then matched with the closest of our measurement dates. The various growth parameters (cumulated stem diameter growth, growth rate, start and end dates) were then analyzed with Student's t-tests to test the effects of the year and of the health status of the trees. Additionally, annual Basal Area Increments (BAI) were computed from the radial growth data for 2011 and 2012, and compared with Student's t-tests.

The effects of health status on functional foliar characteristics were determined by Student's t-tests performed separately on each sampling date. The effect of health status and year of measurement on carbon and nitrogen reserve concentrations was determined by Student's t-tests. A potential difference of reserve variations between the two years of

measurement was tested by creating an annual synthetic variable: the difference between concentrations at budburst and at yellowing for each compound in each organ. On this synthetic variable, we applied paired Student's t-tests to test for a year effect. The same technique was used to test for a possible interaction between health status and phenological stage that could result in differences in reserve dynamics between healthy and declining trees. The relationship between growth and reserve concentrations and dynamics were tested with Pearson correlation tests.

The different parameters of the vulnerability curves (P_{12}, P_{50}, P_{88} and the slope) were analyzed with Student's t-tests to test for differences between healthy and declining trees. Data were analyzed with the R software package (http://www.r-project.org/). In the text, values are presented as averages ± SE.

Results

Stem radial growth

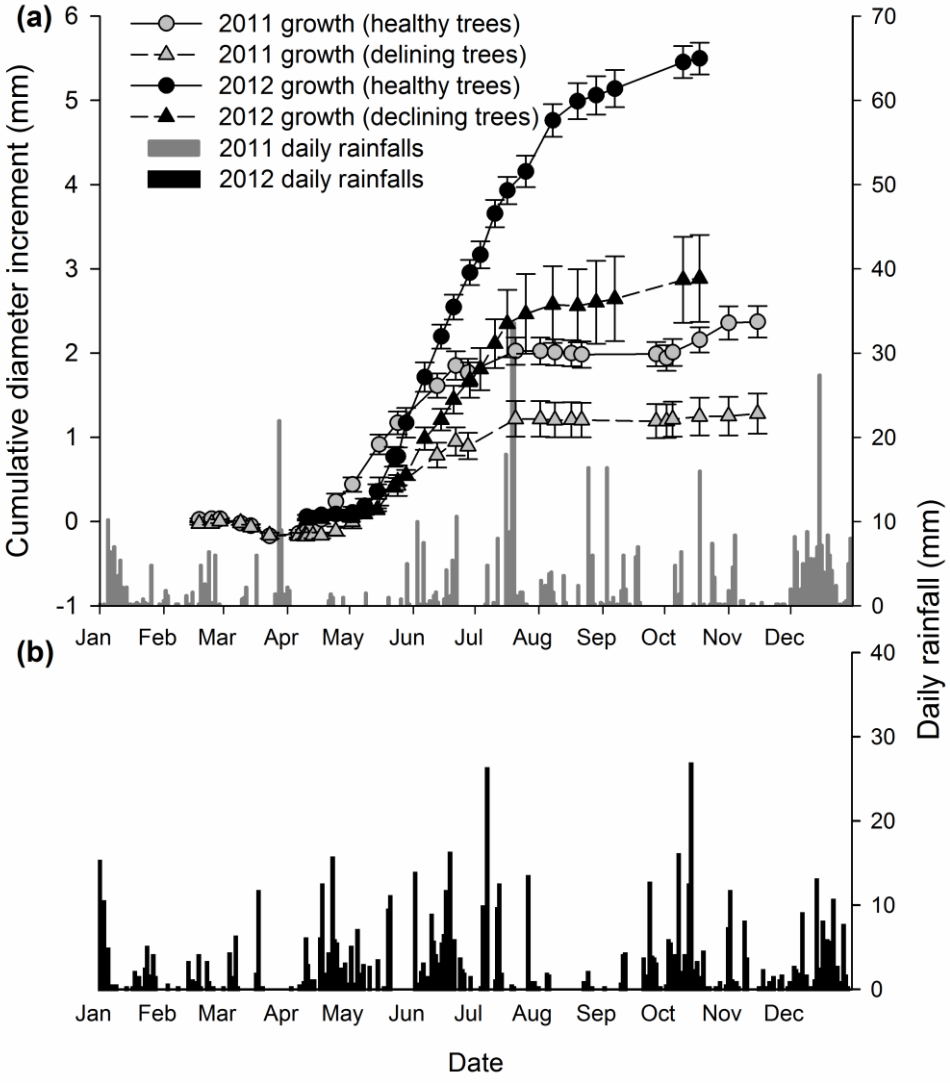

Figure II.1 Cumulative diameter increment (mm) of healthy and declining *Fagus sylvatica* trees and daily rainfall (mm) in 2011(a) and 2012 (b) in Fontainebleau state forest (France). Error bars represent SE (n=5).

Declining trees had lower diameter increments (P<0.05, Figure II.1a) and lower BAI (Basal Area Increment, data not shown) than healthy trees during both years. In 2011, the diameter increment was on average 2.4 mm in healthy trees, and 1.3 mm in declining trees. The 2012 diameter increments were more than twice those in 2011: 5.7 mm in healthy trees

and 3.0 mm in declining trees. Lower growths in declining trees were related to lower growth rates (P<0.05) and a growth duration difference in 2012 (Figure II.1a): declining trees stopped growing 16 days earlier than healthy ones that year (P< 0.05). Stem radial growth in 2012 was higher than the previous year in both healthy and declining trees (P<0.01, Figure II.1a). The dates of the onset of growth were not significantly different between declining and healthy trees in both years, however, trees stopped growing earlier in 2011 (P<0.01), resulting in a shorter growing season than in 2012 (73 days in 2011 for both healthy and declining trees, and 121 and 102 days in 2012 for healthy and declining trees respectively, P<0.01, Figure II.1a).

Leaf characteristics

Health status	Phenological stage	$\delta^{13}C$ of total organic matter (‰)	% N	Mean leaf surface (cm^2)	LMA ($g\ m^{-2}$)	Stomatal densities (stomata m^{-2})
Healthy trees	Budburst	-26.05±0.33	3.24±0.17	5.0±0.5	56.2±0.6	189±19
	Leaf maturity	-28.91±0.20	2.13±0.17	16.0±1.7	101.0±7	
	Yellowing	-29.96±0.27	1.60±0.05	20.0±1.5	72.5±8.1	
Declining trees	Budburst	-26.23±0.26	2.83±0.08	4.7±0.7	54.4±1.9	185±2
	Leaf maturity	-28.87±0.16	2.38±0.13	17.0±2.4	95.4±2.3	
	Yellowing	-29.73±0.25	1.67±0.05	17.4±1.5	81.6±1.1	

LMA: Leaf Mass per Area, % N: percentage of nitrogen in total organic matter. Values are means ± SE

Table II.1 Mean characteristics (± SE) of light-exposed leaves of healthy and declining *Fagus sylvatica* trees in Fontainebleau state forest (France) during the 2012 growing season.

At each studied phenological stage (budburst, leaf maturity, yellowing), all the functional leaf characteristics measured ($\delta^{13}C$ of total leaf organic matter, %C, %N, mean leaf surface and LMA) were similar in healthy and declining trees (Table II.1).

C and N reserves

In 2011, no effect of health status on TNSC concentrations was detected at budburst in the 2010 growth units. However, at yellowing, TNSC concentrations were lower in declining trees than in healthy ones ($P<0.05$, Figure II.2a). No effect of health status on TNSC concentrations in the trunk phloem and xylem (Figure II.2b and c), and in coarse roots (data not shown), was noticed on any date. No effect of health status on starch concentrations at budburst was detected in the 2010 growth units. However, at yellowing, starch concentrations were lower in declining trees than in healthy ones ($P<0.01$, Figure II.2a). No effect of health status on starch concentrations in the trunk phloem and xylem (Figure II.2b and c), or in coarse roots (data not shown), was observed on any date. Health status had no significant effect on soluble sugar concentrations in the 2010 growth units at any phenological stage. No effect of health status on soluble sugars in the trunk phloem was detected at budburst, however, at yellowing, soluble sugars were lower in the trunk phloem of declining trees than in that of healthy ones ($P<0.05$, Figure II.2b). Declining trees had higher soluble sugar concentrations in the xylem than healthy trees at budburst ($P<0.05$), a difference which had disappeared by yellowing (Figure II.2c).

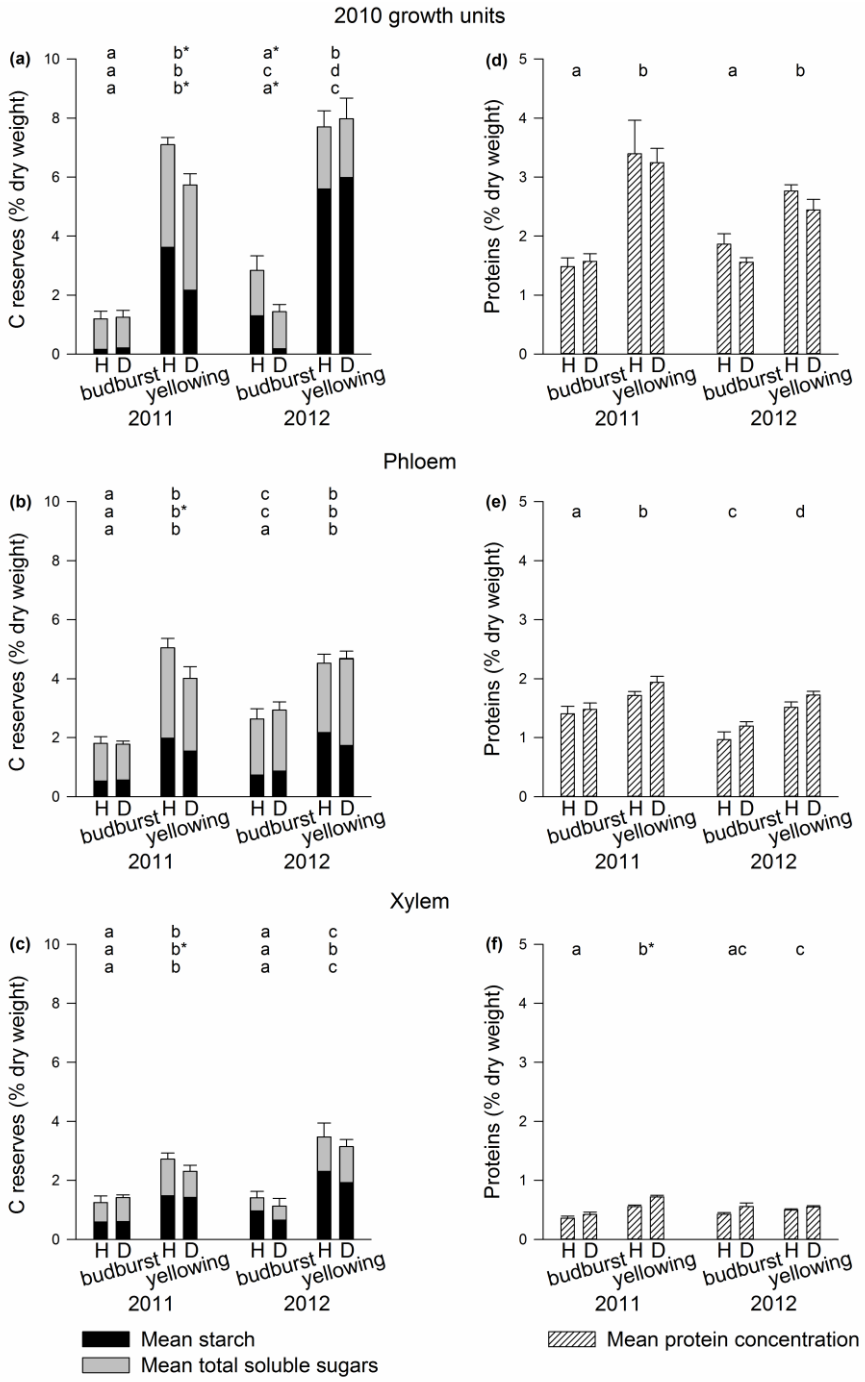

Figure II.2 Concentrations of carbon (soluble sugar and starch, a, b, c) and nitrogen reserves (soluble proteins, d, e, f) in healthy (H) and declining (D) *Fagus sylvatica* trees in Fontainebleau state forest (France) during the 2011 growth season: 2010 growth units (a, d); phloem (b, e); xylem (c, f). The error bars represent SE. The letters correspond to the results of pairwise Wilcoxon-Mann-Whitney (WMW) sum rank tests. Different letters indicate significant differences in average values over all studied trees between dates (P<0.05); in (a), (b) and (c) for TNSC concentrations (upper line), for soluble sugar concentrations (middle line) and for starch concentrations (lower line), in (d), (e), and (f) for protein concentrations. At each date, significant differences (WMW tests) between healthy and declining trees are noted by '*' (P<0.05) for TNSC concentrations (upper line), for soluble sugar concentrations (middle line), and starch concentrations (lower line).

In 2012, declining trees had lower TNSC concentrations in the 2010 growth units than healthy trees at budburst (P<0.05, Figure II.2a), but no such difference was observed at yellowing. Health status had no significant effect on TNSC concentrations in the trunk phloem and xylem (Figure II.2b and c) and in the coarse roots (data not shown) whatever the date. No effect of health status on starch and soluble sugar concentrations was observed in any organ on any date (Figure II.2a, b and c).

Nitrogen reserve concentrations were similar for healthy and declining trees, except at yellowing in 2011 in the xylem, where protein concentrations were lower in healthy than in declining trees (P<0.05, Figure II.2f).

Interannual variations

Globally, TNSC concentrations were higher in 2012 than in 2011 in the 2010 growth units, at both budburst and yellowing (P<0.05, Figure II.2a). TNSC concentrations in the phloem were also higher in 2012 than in 2011 at budburst (P<0.01, Figure II.2b), but were similar at yellowing in both years. No difference was noted between TNSC concentrations in the xylem at budburst in 2011 and 2012, however, those at yellowing were higher in 2012 than in 2011 (P<0.05, Figure II.2c). TNSC concentrations in coarse roots did not differ between 2011 and 2012. The interannual variations of starch concentrations were similar to those of TNSC (Figure II.2a, b and c). Soluble sugar concentrations in the 2010 growth units were higher at budburst in 2012 than in 2011 (P<0.01), but at it was the opposite at yellowing: the soluble sugar concentrations were higher in 2011 than in 2012 (P<0.01, Figure II.2a). In the phloem, the soluble sugar concentrations were higher in 2012 than in 2011 at budburst (P<0.001), but they were not different between years at yellowing (Figure II.2b). In the xylem, at budburst, the soluble sugar concentrations were higher in 2011 than in 2012 (P<0.05), but this difference had disappeared at yellowing (Figure II.2c). Soluble sugar

concentrations in the coarse roots did not significantly differ between 2011 and 2012 (data not shown).

Soluble protein concentrations in the 2010 growth units did not significantly differ between 2011 and 2012 at budburst, whereas they were higher at yellowing in 2011 than in 2012 ($P<0.05$, Figure II.2d). Soluble protein concentrations in the trunk phloem were higher in 2011 than in 2012 at both budburst and yellowing ($P<0.05$, Figure II.2e). Those in the xylem were higher in 2012 than in 2011 at budburst ($P<0.05$), but at yellowing the opposite held true ($P<0.01$, Figure II.2f). In the coarse roots, soluble protein concentrations were higher in 2012 than in 2011 (data not shown).

Considering all the trees, the relationship between stem radial growth and seasonal variations in C reserves differed between years: the replenishment of starch reserves in the 2010 growth units was positively correlated with stem radial growth ($R^2 = 0.50$, $P<0.05$) in 2011, but not in 2012 (Figure II.3). A similar correlation was found for replenishment of TNSC in the stem xylem and stem radial growth ($R^2= 0.39$, $P<0.05$). The seasonal variations of the C reserves were not significantly correlated between years, with the exception of starch in the xylem ($P<0.01$, $R^2= 0.66$). Stem radial growth was significantly correlated between years ($P<0.01$, $R^2= 0.70$).

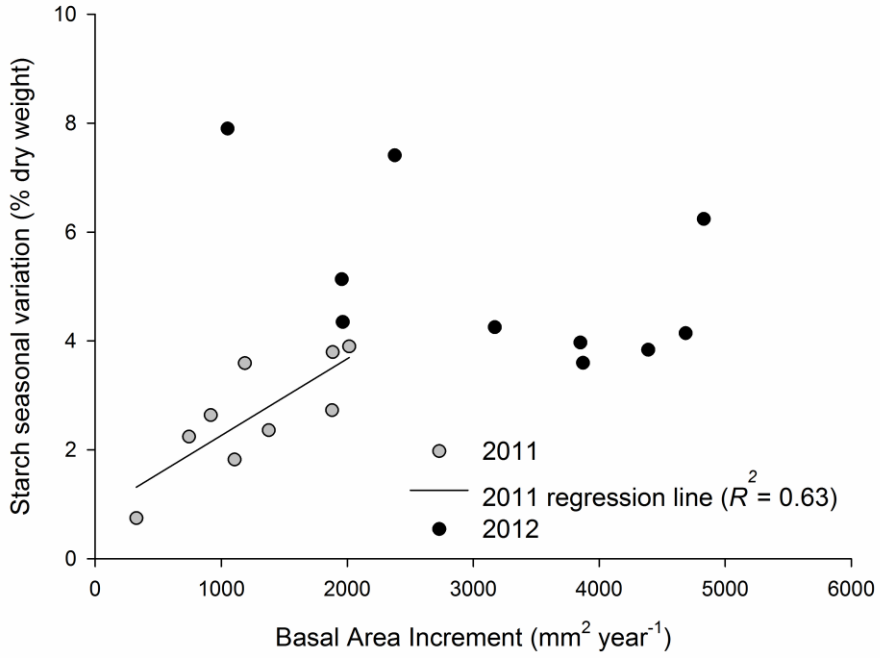

Figure II.3 Relationship between Basal Area Increment (BAI, mm^2 $year^{-1}$) and seasonal variations in starch concentration in the 2010 growth units (% dry weight, calculated as the difference between the starch concentrations at yellowing and budburst), in 2011 (grey dots) and 2012 (black dots). A simple regression line for 2011 is shown.

Hydraulic characteristics

Tree water status

		Pre-dawn water potential (MPa)	Midday water potential (MPa)
June 2011	Healthy trees	-0.26±0.08	
	Declining trees	-0.14±0.04	
August 2011	Healthy trees	-0.22±0.07	
	Declining trees	-0.08±0.01	
May 2012	Healthy trees	-0.23±0.03	-1.29±0.06
	Declining trees	-0.17±0.02	-1.41±0.07
August 2012	Healthy trees	-0.16±0.03	-2.21±0.11
	Declining trees	-0.16±0.03	-2.12±0.11

Table II.2 Average (± SE) pre-dawn and midday water potentials of healthy and declining beech trees in Fontainebleau state forest in June and August 2011 and May and August 2012.

Health status had no significant effect on pre-dawn water potential whatever the sampling year and date. The average midday water potential was significantly lower in August 2012 (-2.1 MPa) than in May (-1.4 MPa, $P<0.0001$, Table II.2).

Branch xylem embolism

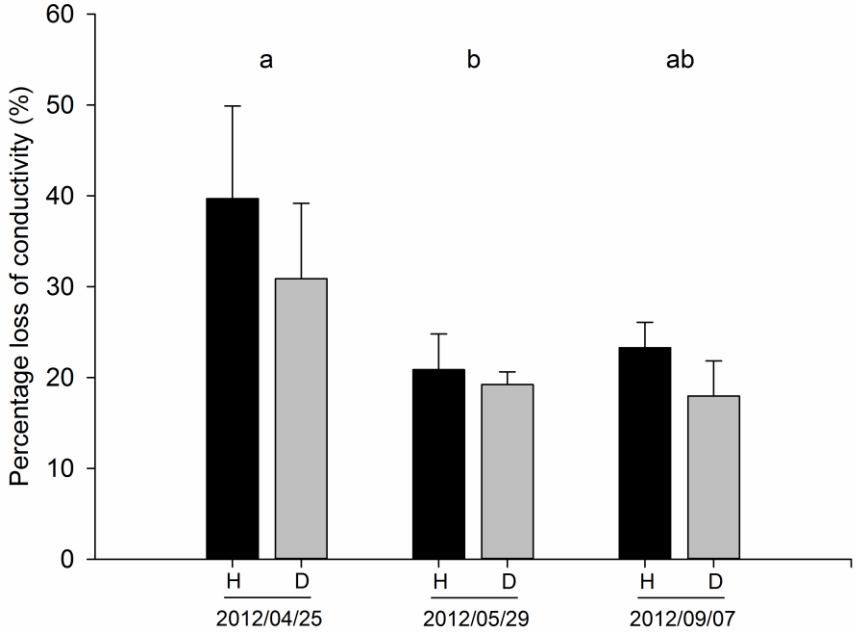

Figure II.4 Average percentage loss of conductivity (± SE) in young branches (1-2 years old) of healthy (H, black) and declining (D, grey) *Fagus sylvatica* trees in Fontainebleau state forest (France) during the 2012 growing season. Letters correspond to the results of Wilcoxon-Mann-Whitney (WMW) sum rank tests. Different letters indicate significant differences between dates (P<0.05).

Healthy and declining trees had similar PLCs at all sampling dates (P<0.05, Figure II.4). Between April 25th and May 29th, the PLC in both groups decreased significantly, from 35.5% to 20.1% (Figure II.4), but it did not show any significant variation in September. Regarding vulnerability to water-stress induced cavitation, the parameters describing the curves did not differ significantly between healthy and declining trees (P>0.05). Average values, considering all trees, were P_{12} -2.3 MPa, P_{50} -3.0 MPa, and P_{88} -3.6 MPa, and the average slope of the linear part of the curve was 87.3 PLC MPa^{-1} (Figure II.5).

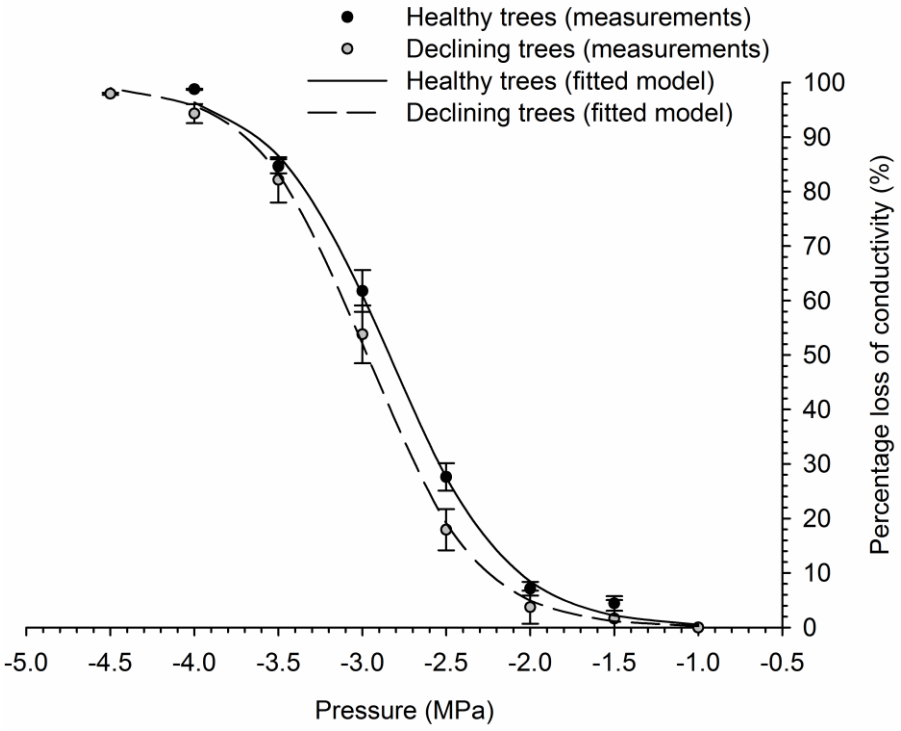

Figure II.5 Average cavitation vulnerability curves of healthy and declining *Fagus sylvatica* trees. The symbols represent the measured values of percentage loss of conductivity (± SE, n=5), and the dashed lines represent the model fitted as described in the Materials and Methods section.

Discussion

How does health status affect stem radial growth and its response to climatic conditions, at the seasonal and interannual scales?

Greater growth rates and a longer growing season in 2012 led to increased growth compared to 2011, in healthy and declining trees. The stem radial growth differences can be explained by climatic conditions: in 2012, precipitation events were frequent and evenly distributed (Figure II.1b), leading to high water availability throughout the growing season, whereas 2011 was marked by a dry spring (Figure II.1a) which probably imposed a hydric limitation on growth. Indeed, it has long been demonstrated that low water availability can impose growth limitations via a decrease in turgor pressure: Hsiao (1973), reviewing the response of plants to water stress, already described this effect more than 40 years ago. More

recently, Zweifel et al. (2006), investigating the intra-annual growth patterns of different tree species (notably beech) growing on dry sites in the Alps, showed the importance of the drought sensitivity of turgor-related cell expansion in intra-annual stem radial growth patterns.

As expected, we observed significantly less growth in declining trees than in healthy ones. Numerous studies have presented similar observations, sometimes long before the appearance of crown-level symptoms : studying silver fir dieback in Slovenia, Torelli et al. (1986) noted that declining trees exhibited narrower rings at least 10 years before the occurrence of the first crown-level symptoms of decline. Jenkins and Pallardy (1995), Pedersen (1998) and Demchik and Sharpe (2000) made similar observations of north-American oak species experiencing high mortality following recurrent droughts: growth decreased up to 20 years before mortality. More recently, Bréda and Badeau (2008) showed in a meta-analysis that following severe droughts, the stem radial growth of some trees was permanently decreased. Even when the decline or mortality event cannot be related exclusively to dry conditions, affected trees can show decreased growth, as shown by Amoroso et al. (2012) in a study of declining *Austrocedrus chilensis*. The decreased annual growth of declining trees we observed is consistent with data already obtained from other trees at the same site (Silva 2010), which showed that currently declining trees had exhibited a decreased stem radial increment since 1959.

The average growth difference between healthy and declining trees was greater in 2012 than in 2011. Declining trees thus seem to have a weaker response to interannual climatic variations than healthy trees. Silva (2010) also observed that declining trees displayed reduced interannual variations since the dry year of 1996, when crown-level symptoms of decline appeared. Pedersen (1998) observed a similar reduction of interannual growth variations during the years preceding tree death in North American oaks. However, the long term studies

of Amoroso et al. (2012) and Klein et al. (2014) showed the opposite trend: declining trees showed a stronger response to interannual climatic variation than healthy trees.

Is the growth reduction associated with altered foliar functioning?

The LMA, percentage of N, $\delta^{13}C$ of total organic matter and stomatal densities were all similar in healthy and declining trees. The observed phenological variations of the measured parameters were consistent with those reported in the literature (Wang et al. 2008; Fotelli et al. 2009; Maunoury-Danger et al. 2010).

Taken together, these parameters give us a partial image of the foliar functioning of the two groups. The $\delta^{13}C$ of the total leaf organic matter is known to be related to the tree's water use efficiency (Farquhar et al. 1989). Combined with the stomatal densities, N content, and the pre-dawn and midday water potentials, it gives us a proxy for stomatal conductance, which is probably similar in healthy and declining trees. This result is not in agreement with observations made at the canopy level in *Populus tremuloides* by Anderegg et al. (2014): aspen canopies in healthy plots had higher stomatal conductance than the canopies in declining plots. However, this apparent contradiction could stem from differences in spatial scales: while Anderegg et al. (2014) studied aspen decline at the plot level, we focused on individual trees growing in the same plot. It would therefore be highly interesting to apply Anderegg et al.'s (2014) approach to healthy and declining beech plots to enable a proper comparison. Concerning the photosynthetic apparatus, the LMA and N contents of leaf organic matter are known to be related to the biochemical leaf photosynthetic capacities (Evans 1989; Poorter et al. 2009). As a consequence, the C assimilation capacity at leaf level is likely to be similar in healthy and declining trees. Thus the observed growth reduction in declining trees is probably not associated with altered leaf functioning but rather with a

reduction of carbon uptake due to a reduced leaf area per tree (indirectly quantified here by the crown loss index), as demonstrated in poplars by Anderegg et al. (2014).

Is altered seasonal growth in declining trees related to a dysfunction in reserve mobilization and/or storage?

There is a lack of information concerning the nitrogen relations of declining trees. The only studies relevant to this subject in the literature deal with soil nitrogen absorption and partitioning by trees during experimental droughts (Fotelli et al. 2002; Fotelli et al. 2004; Geßler et al. 2004; Peuke and Rennenberg 2004). In our study, health status had little effect on N reserve concentrations and dynamics. The measured protein concentrations were within the ranges reported by Bazot et al. (2013) and Gilson et al. (2014) for temperate oaks. Similarly, the percentage of N in sun-exposed leaves was similar in healthy and declining trees, and within the range reported in the literature (Påhlsson 1992; Bussotti et al. 2005). N limitation is therefore unlikely at our study site, and is probably not significantly implicated in beech decline. The results from literature are rather contradictory, and are not directly comparable to those presented in this paper. Even during the moderate drought year 2011, leaf N and protein contents were similar in declining and healthy trees, an observation consistent with previous results. Indeed, Fotelli et al. (2002) found no effect of drought on the protein nitrogen content of beech seedlings. Moreover, Fotelli et al. (2004) and Peuke and Rennenberg (2004) observed no effect of drought on nitrogen assimilation in beech seedlings. However, Fotelli et al. (2002) and Geßler et al. (2004) showed that under dry conditions, nitrogen absorption by beech trees was reduced.

Young branches of declining trees had less C reserves than healthy ones following the constraining 2011 growing season, a reserve deficit caused by lower starch concentrations in the declining trees. A reduction of C starch concentrations following a drought was

documented in the stems of beech trees that had suffered defoliation during the 2003 heat wave and drought (Bréda et al. 2006). Similar observations were made in Mediterranean forest trees: a clear association between drought-induced defoliation and lower C reserve concentrations in *Quercus ilex* was observed (Galiano et al. 2012; Rosas et al. 2013). In our study, this difference in C reserve concentrations was still present at the following budburst. However, after the moist 2012 growing season, C reserve concentrations in young branches of healthy and declining trees were similar (Figure II.2a). It therefore seems that in 2012, declining trees made a functional adjustment at the tissue level by storing more C reserves in young branches than healthy trees. The reduced stem radial growth of declining trees was therefore associated with differences in reserve concentrations between budburst and yellowing. This relationship between growth and C reserves can be examined more precisely by correlating stem radial growth and reserve replenishment of individual trees during the two contrasted growing seasons. In 2011, stem radial growth was positively correlated with the replenishing of C reserves in young branches (Figure II.3): the more a tree grew, the greater the increase of C reserve concentrations during the growing season. This correlation was however not significant in 2012. Declining trees in fact increased their stem radial growth between 2011 and 2012 to a lesser extent than healthy trees, while increasing the replenishment of their C reserves in stems and young branches between the two years. In beech, C reserve storage and growth were concomitant for both healthy and declining trees in 2011 and 2012 (data not shown). Barbaroux and Bréda (2002) and Scartazza et al. (2013) similarly showed that in beech, growth and C reserve formation were not disconnected. Nevertheless it was noticeable that in 2012, the growth of declining trees stopped almost two weeks before that of healthy trees (Figure II.1a; beginning of August in declining trees and mid-August in healthy trees). Net C assimilation at the tree level has been shown to occur from May to September in beech (Lebaube et al. 2000; Pilegaard et al. 2001). Thus, the

assimilated C could be stored after the cessation of growth, explaining the higher increase of C reserve concentrations in branches of declining trees. The concomitance of growth and C storage at the beginning of the growing season confirmed the active nature of C reserve formation, as described by Chapin et al. (1990), whereas C storage is likely to become a passive process after the cessation of growth. However, if the stem radial growth of declining trees was actively down regulated, C reserve formation at the end of the 2012 growing season could be considered semi-active, as described by Dietze et al. (2014).

Is water transport altered by health status during the growing season?

Similar pre-dawn water potentials in healthy and declining trees in both 2011 and 2012 indicate that the trees probably have similar access to soil water resources (Ritchie and Hinckley 1975). This finding is consistent with that presented by Silva (2010): the local variability of soil depth around beech trees growing in the same plot (as in this paper) was not spatially correlated with the pattern of decline. Our results showed similar native embolism levels throughout the growing season in healthy and declining trees, well below levels lethal to beech (PLC around 90% Barigah et al. 2013). Moreover, healthy and declining trees also had similar vulnerabilities to cavitation, well within the range reported for beech (Wortemann et al. 2011). As a consequence, our data suggest that hydraulic failure *sensu stricto* (without considering the potential interactions with C metabolism) is probably not a chronic functional mechanism involved in beech decline in Fontainebleau state forest. However, hydraulic failure during an extreme dry year such as 2003 leading to observed loss of young branches in declining trees cannot be excluded. In this species, the xylem function seems to fail only under very prolonged and extreme hydric stress (Barigah et al. 2013, study on saplings).

Potential interaction between C and hydraulics

Sala et al. (2012) exposed that the hydraulic and C systems of trees are likely to interact with each other. Since declining trees had less C reserves than healthy ones in young branches at the end of the constraining 2011 growing season, it is possible that, in the case of a more severe drought, or of recurrent droughts, declining trees could fail to maintain sufficient C reserve concentrations in other storage compartments in order to ensure the beginning of the following growing season, and to ensure proper osmoregulation of the xylem sap, and consequently suffer from C-related hydraulic failure. Indeed, the importance of sufficient C reserve concentrations in the maintenance of the xylem transport via osmoregulation processes has recently been demonstrated: Sevanto et al. (2014) showed that under drought conditions, hydraulic failure in *Pinus edulis* might be associated with a loss of C reserve content required for osmoregulation, in turn promoting hydraulic failure. A study of within-crown variability of both C reserves and hydraulic function could clarify the link between these two aspects of tree function, and improve our understanding of the increased fine branch mortality observed in declining trees.

In conclusion, this study documents tree functional responses after a long-term, drought-related decline in beech, at the individual level. Declining trees were characterized by growth clearly lower than that of healthy trees. This decrease is linked to reduced carbon assimilation, mainly due to a thinned canopy rather than an impairment of leaf function. During a favorable year following a constraining one, their growth recovery was limited especially by the length of the growing season. This apparent limitation could in fact be beneficial, by allowing a seasonal recovery of the reserve content in wood tissue. With the exception of growth, functioning of declining trees is globally still close to that of healthy trees for the surviving tissues (for C, N and hydraulic functionning). This study concerned favorable or moderately stressful years, and should be completed by examining tree functioning during an extremely

constraining year, when hydraulic and reserve dynamics are probably impacted to greater and different extents.

Acknowledgements

The authors would like to acknowledge the students who contributed to data collection and field work: Alain Sévéré for his help with the branch sampling, and Michèle Viel and Patricia Le Thuaut for technical assistance. We are grateful to the French National Forest Office (ONF) for allowing us to carry out these experiments. The authors thank Stéphane Herbette (UMR PIAF INRA, Université Blaise Pascal), for help with the measurements of vulnerability curves.

Funding

AD's doctoral grant was provided by the French Ministry of Higher Education and Scientific Research. Additional financial support was provided by a CYTRIX project (EC2CO) funded by CNRS and INSU and also by the ESE laboratory supported by the University Paris Sud, CNRS and AgroParisTech.

References

Adams HD, Germino MJ, Breshears DD, et al. (2013) Nonstructural leaf carbohydrate dynamics of Pinus edulis during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. New Phytol 197:1142–1151. doi: 10.1111/nph.12102

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, et al. (2009) Reply to Sala: Temperature sensitivity in drought-induced tree mortality hastens the need to further resolve a physiological model of death. Proc Natl Acad Sci U S A 106:E69. doi: 10.1073/pnas.0905282106

Allen CD, Macalady AK, Chenchouni H, et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684. doi: 10.1016/j.foreco.2009.09.001

Amoroso MM, Daniels LD, Larson BC (2012) Temporal patterns of radial growth in declining Austrocedrus chilensis forests in Northern Patagonia: The use of tree-rings as an indicator of forest decline. For Ecol Manage 265:62–70.

Anderegg WRL, Anderegg LDL, Berry JA, Field C (2014) Loss of whole-tree hydraulic conductance during severe drought and multi-year forest die-off. Oecologia 175:11–23. doi: 10.1007/s00442-013-2875-5

Anderegg WRL, Berry JA, Smith DD, et al. (2012) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. Proc Natl Acad Sci U S A 109:233–237.

Bahrman N, Plomion C, Petit RJ, Kremer A (1997) Contribution of two-dimensional electrophoresis of proteins to maritime pine genetics. Ann For Sci 54:225–236.

Barbaroux C, Bréda N (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. Tree Physiol 22:1201–1210.

Barbaroux C, Bréda N, Dufrêne É (2003) Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (Quercus petraea and Fagus sylvatica). New Phytol 157:605–615. doi: 10.1046/j.1469-8137.2003.00681.x

Barigah TS, Charrier O, Douris M, et al. (2013) Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. Ann Bot 112:1431–1437. doi: 10.1093/aob/mct204

Bazot S, Barthes L, Blanot D, Fresneau C (2013) Distribution of non-structural nitrogen and carbohydrate compounds in mature oak trees in a temperate forest at four key phenological stages. Trees - Struct Funct 27:1023–1034. doi: 10.1007/s00468-013-0853-5

Bossel H (1986) Dynamics of forest dieback: Systems analysis and simulation. Ecol Modell 34:259–288. doi: http://dx.doi.org/10.1016/0304-3800(86)90008-6

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Bréda N, Badeau V (2008) Forest tree responses to extreme drought and some biotic events: Towards a selection according to hazard tolerance? Comptes Rendus Geosci 340:651–662. doi: 10.1016/j.crte.2008.08.003

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought : a review of ecophysiological responses , adaptation processes and long-term consequences. Ann For Sci 63:625–644. doi: 10.1051/forest

Breshears DD, Myers OB, Meyer CW, et al. (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. Front Ecol Environ 7:185–189. doi: 10.1890/080016

Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiol 149:575–584. doi: 10.1104/pp.108.129783

Brummer Y, Cui SW (2005) Understanding Carbohydrate Analysis. Food Carbohydrates Chem Phys Prop Appl. Taylor and Francis group, pp 67–104

Bussotti F, Prancrazi M, Matteucci G, Gerosa G (2005) Leaf morphology and chemistry in Fagus sylvatica (beech) trees as affected by site factors and ozone: results from CONECOFOR permanent monitoring plots in Italy. Tree Physiol 25:211–219. doi: 10.1093/treephys/25.2.211

Chapin SF, Schultze E-D, Mooney HA (1990) The Ecology and Economics of Storage in Plants. Annu Rev Ecol Syst 21:423–447.

Choat B, Jansen S, Brodribb TJ, et al. (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755.

Cochard H (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. Plant, Cell Environ 25:815–819. doi: 10.1046/j.1365-3040.2002.00863.x

Cochard H, Herbette S, Barigah TS, et al. (2010) Does sample length influence the shape of xylem embolism vulnerability curves? A test with the Cavitron spinning technique. Plant Cell Environ 33:1543–1552. doi: 10.1111/j.1365-3040.2010.02163.x

Demchik MC, Sharpe WE (2000) The effect of soil nutrition, soil acidity and drought on northern red oak (Quercus rubra L.) growth and nutrition on Pennsylvania sites with high and low red oak mortality. For Ecol Manage 136:199–207. doi: http://dx.doi.org/10.1016/S0378-1127(99)00307-2

Dietze MC, Sala A, Carbone MS, et al. (2014) Nonstructural Carbon in Woody Plants. Annu Rev Plant Biol 65:667–687. doi: 10.1146/annurev-arplant-050213-040054

Domec J-C, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees - Struct Funct 15:204–214. doi: 10.1007/s004680100095

Durand-Gillmann M, Cailleret M, Boivin T, et al. (2012) Individual vulnerability factors of Silver fir (Abies alba Mill.) to parasitism by two contrasting biotic agents: mistletoe (Viscum album L. ssp. abietis) and bark beetles (Coleoptera: Curculionidae: Scolytinae) during a decline process. Ann For Sci 69:1–15. doi: 10.1007/s13595-012-0251-y

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19. doi: 10.1007/BF00377192

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537.

Fotelli MN, Nahm M, Radoglou K, et al. (2009) Seasonal and interannual ecophysiological responses of beech (Fagus sylvatica) at its south-eastern distribution limit in Europe. For Ecol Manage 257:1157–1164.

Fotelli MN, Rennenberg H, Geßler A (2002) Effects of Drought on the Competitive Interference of an Early Successional Species (Rubus fruticosus) on Fagus sylvatica L. Seedlings: 15N Uptake and Partitioning, Responses of Amino Acids and other N Compounds. Plant Biol 4:311–320. doi: 10.1055/s-2002-32334

Fotelli MN, Rienks M, Rennenberg H, Geßler A (2004) Climate and forest management affect 15N-uptake, N balance and biomass of European beech seedlings. Trees - Struct Funct 18:157–166. doi: 10.1007/s00468-003-0289-4

Galiano L, Martínez-Vilalta J, Lloret F (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. New Phytol 190:750–759. doi: 10.1111/j.1469-8137.2010.03628.x

Galiano L, Martínez-Vilalta J, Sabaté S, Lloret F (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiol 32:478–489. doi: 10.1093/treephys/tps025

Galvez D a, Landhäusser SM, Tyree MT (2011) Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation? Tree Physiol 31:250–257. doi: 10.1093/treephys/tpr012

Geßler A, Keitel C, Nahm M, Rennenberg H (2004) Water Shortage Affects the Water and Nitrogen Balance in Central European Beech Forests. Plant Biol 6:289–298.

Gilson A, Barthes L, Delpierre N, et al. (2014) Seasonal changes in carbon and nitrogen compound concentrations in a Quercus petraea chronosequence. Tree Physiol 34:716–729. doi: 10.1093/treephys/tpu060

Le Goff N, Ottorini J-M (2001) Root biomass and biomass increment in a beech (Fagus sylvatica L.) stand in North-East France. Ann For Sci 58:1–13.

Hacke UG, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in Fagus sylvatica f. purpurea and Populus balsamifera. J Exp Bot 46:1177–1183. doi: 10.1093/jxb/46.9.1177

Hartmann H, Ziegler W, Kolle O, Trumbore S (2013a) Thirst beats hunger - declining hydration during drought prevents carbon starvation in Norway spruce saplings. New Phytol 200:340–349. doi: 10.1111/nph.12331

Hartmann H, Ziegler W, Trumbore S (2013b) Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy. Funct Ecol 27:413–427. doi: 10.1111/1365-2435.12046

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081.

Hsiao TC (1973) Plant Responses to Water Stress. Annu Rev Plant Physiol 24:519–570. doi: 10.1146/annurev.pp.24.060173.002511

Intergovernmental Panel on Climate Change (2014) Climate Change 2014: Synthesis Report.

Jenkins MA, Pallardy SG (1995) The influence of drought on red oak group species growth and mortality in the Missouri Ozarks. Can J For Res 25:1119–1127. doi: 10.1139/x95-124

Klein T, Hoch G, Yakir D, Körner C (2014) Drought stress, growth and nonstructural carbohydrate dynamics of pine trees in a semi-arid forest. Tree Physiol 34:981–992. doi: 10.1093/treephys/tpu071

Lebaube S, Goff N Le, Ottorini J-M, Granier A (2000) Carbon balance and tree growth in a Fagus sylvatica stand. Ann For Sci 57:49–61.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379. doi: 10.1890/06-2057.1

Leuzinger S, Bigler C, Wolf A, Körner C (2009) Poor methodology for predicting large-scale tree die-off. Proc Natl Acad Sci U S A 106:E106. doi: 10.1073/pnas.0908053106

López BC, Gracia CA, Sabaté S, Keenan T (2009) Assessing the resilience of Mediterranean holm oaks to disturbances using selective thinning. Acta Oecologica 35:849–854. doi: http://dx.doi.org/10.1016/j.actao.2009.09.001

Lorenz M, Becher G (2012) Forest Condition in Europe 2012, Technical Report of ICP Forests. Hamburg

Martínez-Vilalta J, Piñol J, Beven K (2002) A hydraulic model to predict drought-induced mortality in woody plants: an application to climate change in the Mediterranean. Ecol Modell 155:127–147. doi: http://dx.doi.org/10.1016/S0304-3800(02)00025-X

Maunoury-Danger F, Fresneau C, Eglin T, et al. (2010) Impact of carbohydrate supply on stem growth, wood and respired CO2 delta13C: assessment by experimental girdling. Tree Physiol 30:818–830. doi: 10.1093/treephys/tpq039

McDowell NG, Beerling DJ, Breshears DD, et al. (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. Trends Ecol Evol 26:523–532. doi: 10.1016/j.tree.2011.06.003

McDowell NG, Pockman WT, Allen CD, et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739. doi: 10.1111/j.1469-8137.2008.02436.x

McDowell NG, Sevanto S (2010) The mechanisms of carbon starvation: how, when, or does it even occur at all ? New Phytol 186:263–264.

Nageleisen L-M, Goudet M (2011) Manuel de Notation des dommages forestiers (symptômes, causes, état des cimes). Paris

Nardini A, Battistuzzo M, Savi T (2013) Shoot desiccation and hydraulic failure in temperate woody angiosperms during an extreme summer drought. New Phytol 200:322–329. doi: 10.1111/nph.12288

Påhlsson AM (1992) Influence of nitrogen fertilization on minerals, carbohydrates, amino acids and phenolic compounds in beech (Fagus sylvatica L.) leaves. Tree Physiol 10:93–100.

Pedersen BS (1998) The role of stress in the mortality of midwestern oaks as indicated by growth prior to death. Ecology 79:79–93.

Peuke AD, Rennenberg H (2004) Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (Fagus sylvatica L.): phosphorus most affected by drought. Trees - Struct Funct 18:639–648. doi: 10.1007/s00468-004-0335-x

Pilegaard K, Hummelshøj P, Jensen NO, Chen Z (2001) Two years of continuous CO2 eddy-flux measurements over a Danish beech forest. Agric For Meteorol 107:29–41. doi: http://dx.doi.org/10.1016/S0168-1923(00)00227-6

Piper FI (2011) Drought induces opposite changes in the concentration of non-structural carbohydrates of two evergreen Nothofagus species of differential drought resistance. Ann For Sci 68:415–424. doi: 10.1007/s13595-011-0030-1

Plaut JA, Yepez EA, Hill J, et al. (2012) Hydraulic limits preceding mortality in a piñon–juniper woodland under experimental drought. Plant Cell Environ 35:1601–1617.

Poorter H, Niinemets Ü, Poorter L, et al. (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 182:565–588. doi: 10.1111/j.1469-8137.2009.02830.x

Poot P, Veneklaas EJ (2012) Species distribution and crown decline are associated with contrasting water relations in four common sympatric eucalypt species in southwestern Australia. Plant Soil 364:409–423. doi: 10.1007/s11104-012-1452-8

Ritchie GA, Hinckley TM (1975) The Pressure Chamber as an Instrument for Ecological Research. Adv Ecol Res Volume 9:165–254. doi: http://dx.doi.org/10.1016/S0065-2504(08)60290-1

Rosas T, Galiano L, Ogaya R, et al. (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Front Plant Sci 4:1–16. doi: 10.3389/fpls.2013.00400

Sala A (2009) Lack of direct evidence for the carbon-starvation hypothesis to explain drought-induced mortality in trees. Proc Natl Acad Sci U S A 106:E68. doi: 10.1073/pnas.0904580106

Sala A, Piper FI, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol 186:274–281. doi: 10.1111/j.1469-8137.2009.03167.x

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiol 32:764–775. doi: 10.1093/treephys/tpr143

Scartazza A, Moscatello S, Matteucci G, et al. (2013) Seasonal and inter-annual dynamics of growth, non-structural carbohydrates and C stable isotopes in a Mediterranean beech forest. Tree Physiol 33:730–742. doi: 10.1093/treephys/tpt045

Sevanto S, Mcdowell NG, Dickman LT, et al. (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell Environ 37:153–161. doi: 10.1111/pce.12141

Silva DE (2010) Ecologie du hêtre (Fagus sylvatica L.) en marge sud-ouest de son aire de distribution. Université Henri Poincaré, Nancy

Sparks JP, Black RA (1999) Regulation of water loss in populations of Populus trichocarpa: the role of stomatal control in preventing xylem cavitation. Tree Physiol 19:453–459. doi: 10.1093/treephys/19.7.453

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell Environ 11:35–40. doi: 10.1111/j.1365-3040.1988.tb01774.x

Torelli N, Čufar K, Robic D (1986) Some wood anatomical, physiological and silvicultural aspects of silver fir dieback in Slovenia (NW Yugoslavia). Iawa Bull 7:343–350.

Urli M, Porté AJ, Cochard H, et al. (2013) Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol 33:672–683. doi: 10.1093/treephys/tpt030

Vitasse Y, Delzon S, Dufrêne É, et al. (2009) Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? Agric For Meteorol 149:735–744. doi: 10.1016/j.agrformet.2008.10.019

Wang Q, Iio A, Tenhunen J, Kakubari Y (2008) Annual and seasonal variations in photosynthetic capacity of Fagus crenata along an elevation gradient in the Naeba Mountains, Japan. Tree Physiol 28:277–285. doi: 10.1093/treephys/28.2.277

Waring RH (1987) Characteristics of Trees Predisposed to Die. Bioscience 37:569–574.

Wheeler JK, Huggett BA, Tofte AN, et al. (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. Plant Cell Environ 36:1938–1949. doi: 10.1111/pce.12139

Wildhagen H, Dürr J, Ehlting B, Rennenberg H (2010) Seasonal nitrogen cycling in the bark of field-grown Grey poplar is correlated with meteorological factors and gene expression of bark storage proteins. Tree Physiol 30:1096–1110. doi: 10.1093/treephys/tpq018

Wiley E, Huepenbecker S, Casper BB, Helliker BR (2013) The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favour of storage? Tree Physiol 33:1216–1228. doi: 10.1093/treephys/tpt093

Wortemann R, Herbette S, Barigah TS, et al. (2011) Genotypic variability and phenotypic plasticity of cavitation resistance in Fagus sylvatica L. across Europe. Tree Physiol 31:1175–1182.

Zhao J, Hartmann H, Trumbore S, et al. (2013) High temperature causes negative whole-plant carbon balance under mild drought. New Phytol 200:330–339. doi: 10.1111/nph.12400

Zweifel R, Zimmermann L, Zeugin F, Newbery DM (2006) Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. J Exp Bot 57:1445–1459. doi: 10.1093/jxb/erj125

III. Long term decline in beech is associated to structural change in the stem living tissue with no apparent alteration of reserve use, maintenance respiration or frost resistance.

Long term decline in beech is associated to structural change in the stem living tissue with no apparent alteration of reserve use, maintenance respiration or frost resistance.

Alice Delaporte[123*]; Stéphane Bazot[123], Anne-Violette Lavoir[4]; Thierry Améglio[56]; Philippe Rozenberg[7];Claire Damesin[123]

1. Université Paris-Sud, UMR 8079, Laboratoire Ecologie Systématique et Evolution, Orsay, F-91405, Orsay, France

2. CNRS, UMR 8079, Laboratoire Ecologie Systématique et Evolution, Orsay, F-91405, Orsay, France

3. AgroParisTech, UMR 8079, Laboratoire Ecologie Systématique et Evolution, F-75231 Paris, France

4. Université de Nice Sophia-Antipolis (UNS), UMR1355, 400 Route des Chappes, 06903, Sophia-Antipolis, France

5. INRA, UMR547 PIAF, Clermont-Ferrand, France

6. Clermont Université, Université Blaise Pascal, UMR547 PIAF, Clermont-Ferrand, France

7. INRA Val de Loire, UR0588, Unité d'Amélioration Génétique et Physiologie Forestières, 2163 Avenue de la Pomme de Pin, CS 40001 Ardon, 45075, Orléans Cedex

* Corresponding author:

Email: alice.delaporte@u-psud.fr

Telephone number: 33-1 69 15 56 95

Abstract

Key message This study documents the stem ecophysiology during the non growing season in a context of tree decline, and provides evidence of structural change of the living tissue.

Abstract Tree decline is an increasing problem associated with changing climate. However, gaps in our knowledge of the functional mechanisms underlying tree decline prevent relevant predictions on future tree health. In particular, the ecophysiological processes determining tree survival in winter remain poorly documented. In this paper, we investigate several aspects of tree functioning during winter in the aboveground woody compartment of healthy and declining beech (*Fagus sylvatica* L.) trees. Trees of contrasted health status showed very close frost hardiness and maintenance respiration rates. Carbon (soluble sugars and starch) and nitrogen (soluble proteins and amino acids) reserve concentrations and dynamics were also mostly similar. However, the health status of the trees was associated with structural differences in the storage tissue. Indeed, declining trees had a greater proportion of radial parenchyma in the xylem than healthy trees, leading to less efficient reserve storage in declining tree per unit of parenchyma area. Moreover, the shape of parenchyma rays was different between healthy and of declining trees, as indicated by a significantly higher ray width/ray length ratio in declining trees compared to healthy ones. This difference in living tissue anatomy was correlated to ring widths and wood densities, suggesting the existence of structural constraints on the formation of parenchyma rays imposed by growth characteristics These structural features could alter the functional connexion between the parenchyma cells and the conducting tissues (xylem and phloem), hindering the ability of the tree to recover from embolism and/or impact the remobilisation of reserves. These results suggest that functional anatomy could be an interesting approach to better understand the mechanisms underlying tree decline and morality.

Keywords Tree decline; Winter; Aboveground woody compartment; Maintenance respiration; Frost hardiness; Radial parenchyma

Introduction

Tree decline consists in a set of symptoms resulting from complex associations of biotic and abiotic stressors (Manion 1981). Some of these stressors might, in certain regions, be enhanced by global change. For instance, droughts and pest-related damage in temperate climates might become more intense and more frequent (Lindner et al. 2010; Intergovernmental Panel on Climate Change 2014). Despite this probable increase of short-term stressors, their overall impact on tree health and vigour in the long term remains unclear. To reduce this uncertainty, it is necessary to develop a better understanding of the functional mechanisms underlying tree decline and mortality (Bréda and Badeau 2008; Sala et al. 2010). A growing number of studies have investigated the ecophysiological responses of trees to moderate or severe droughts. Our current understanding of drought-related tree decline and mortality is that it probably involves imbalances and/or malfunction in the storage and transport systems of water and carbon (C) reserves (McDowell et al. 2008; Sala et al. 2010; McDowell 2011). However, studies of tree decline and mortality in temperate climate mostly deal with the processes occurring during the spring and summer (referred to here as the active season) (Anderegg et al. 2014, Montwé et al. 2014, Zang et al. 2014, Moulinier et al. 2014, Saffell et al. 2014, among others).

In temperate climates, species have to use conservative physical and physiological strategies to survive during winter (Kreyling 2010). An example of such physical conservative strategies is the deciduous behavior of some woody species (Kreyling 2010). In deciduous species, the absence of leaves during the several months of winter implies that trees rely on their C reserves to maintain their living tissues (Chapin et al. 1990). In tree stems, C reserves are stored in parenchyma cells (Tromp 1983). The volume of parenchyma in the xylem and phloem is considered a good estimate of the potential maximum storage capacity of the tree (Spicer and Holbrook 2007). Additionally, parenchyma in the xylem forms a three-

dimensional lattice, and play a crucial role in the remobilization and translocation of C reserves (Spicer 2014). An alteration of the reserve remobilization and translocation processes could hinder tree survival in the long term (Sala et al. 2010). Moreover, a potential association between tree vitality and parenchyma ray abundance has been recently suggested (Fonti et al. 2015). The volume and functional state of parenchyma cells could therefore play an important role in a context of tree decline. It can be hypothesized that declining trees could have less parenchyma in the xylem, meaning that their potential maximum storage capacity would be lower than the one of healthy trees, but also that their ability to remobilize and redistribute reserve compounds could be altered.

The roles played by parenchyma imply that they must be physiologically active. As such, they require maintenance, i.e. a minimum metabolism has to be kept to ensure the turn-over of enzymes and the integrity of the cellular structure (Ryan 1990). During winter, tissue maintenance also includes the maintenance of soluble sugar and other metabolite and/or ion gradients to ensure cold hardening (Ögren 2000). Maintenance respiration provides the energy to fuel these processes (Penning de Vries 1975). Internal factors impacting the intensity of maintenance respiration include the physiological state of the organ studied and the soluble sugar concentrations (Ögren 2000). For woody stems, the maintenance respiration rate has been related to the N content of the xylem (Ceschia et al. 2002) and the quantity of living cells in the xylem (Ceschia et al. 2002). If declining trees have less living tissue in the xylem than healthy ones, as hypothesized above, a lower maintenance respiration rate could be expected.

Maintenance respiration uses C reserves as substrates to produce energy. C reserves, and particularly soluble sugars, are thus crucial components for trees during winter, as they act as energy sources for maintenance respiration (Ögren 2001). The carbon relations of trees during winter therefore have potentially strong implications for their long-term survival. Furthermore, C and N reserves provide energy and materials during the growth flush early in

spring, until the new leaves reach autotrophy (Barbaroux and Bréda 2002). The ability to store and remobilize C and N reserves therefore determines the ability of the tree to resume growth in spring (Tromp 1983). A modification of the ability of the tree to store and/or remobilize C and N reserves could thus also have a negative impact on the spring flush. Tree decline is potentially associated with altered C reserve storage and/or remobilization processes (McDowell et al. 2008; Sala et al. 2010). More precisely, the reduced leaf area of declining trees, potentially associated with limited water transport, can lead to drastic reductions of whole-tree photosynthetic C assimilation (Anderegg et al. 2014). In parallel, processes related to phloem loading and transport could be negatively affected, leading to a reduced ability to remobilize stored reserves (Sala et al. 2010; McDowell 2011). So far, the C reserve concentrations and dynamics have been investigated mainly during the active season, but reserve use in winter for maintenance processes has seldom, if ever, been specifically assessed.

Carbohydrates, and more precisely soluble sugars, have also a direct and major role in the cold hardening process (Sakai 1962). More precisely, sucrose acts as a stabilizing agent for cellular membranes by direct interaction with lipids, preventing mechanical damage linked with dehydration when most of the liquid water is located outside the cell (Charrier and Améglio 2011). Sufficient amounts C reserve are therefore essential for proper cold acclimation (Charrier and Améglio 2011). Additionally, some amino acids and proteins also contribute to cold hardening (Janská et al. 2010). Moreover, the positive correlation between whole-plant N content and cold hardiness observed by Oliet et al. (2013) suggests a potential role of N reserve compounds in winter survival. Altered C and N reserve amounts and/or dynamics, could therefore impact the cold hardening of declining trees, making them more prone to frost-induced damage than healthy trees.

Despite their potential importance, no study has specifically investigated the processes occurring during the dormant season in temperate trees in a context of long term decline. In this study, we investigated different aspects of stem ecophysiology during the leafless season by addressing the following questions:

i. Is tree decline associated with differences in structure and proportion of living tissue in the stem xylem?

ii. Is the maintenance respiration of these living tissues altered with tree decline?

iii. Is the maintenance of the stem living tissue supported by equivalent C and N reserve concentrations and dynamics in healthy and declining trees?

iv. Is cold hardening of living cells impaired by tree decline?

To answer these questions, stem CO2 efflux, frost resistance, starch, soluble sugar, protein and amino acid contents were compared between declining and healthy beech trees during the non growing season. In parallel, ray anatomical structure and proportion were examined and related to other ring characteristics.

Materials and methods

Site and stand description

The study was carried out in Fontainebleau state forest, France (48°22’ N, 02°36’ E, mean elevation 120 m a.s.l.). This forest extends over 17, 000 ha, 60 km southeast of Paris. The climate is temperate, with a mean annual temperature of 10.6°C and mean annual precipitations of 749 mm, well distributed throughout the year (average on the 1960-2010 period). The soil texture is dominated by Stampian sand mixed with loam and clay at different depths. The study site is a mature, monospecific beech (*Fagus sylvatica* L.) stand, where mean tree age in 2011 was 95 years and dominant height 27 m, showing heavy signs of

decline since the late 1990's. Trees were growing on a rather shallow podzoluvisol soil (mean depth 0.6 m), with a C/N ratio of 18.7 and extractible soil water content of 101 mm.

Experimental design

Five healthy and five declining trees were selected using the DEPEFEU (DEPErissement des FEUillus) protocol (Nageleisen and Goudet 2011), developed by the French National Forest Office (Office National des forêts, ONF). These two categories corresponded to trees with dense crowns and abundant fine ramification (healthy trees), and with very transparent crowns, only one or two main branches remaining and very limited fine ramification (declining trees). On these trees, yellowing (50% of senescing leaves in the upper third of the crown) and budburst (50% of broken buds in the upper third of the crown) were monitored as described in Delaporte et al (2015, submitted).

During the 2011 dormant season, stem maintenance respiration was measured at 4 different dates (2011/02/18, 2011/03/11, 2011/03/16, 2011/03/25), corresponding to increasing ambient temperatures. C and N reserves were measured at yellowing and in the middle of winter in 2011 (2011/11/02 and 2011/12/14 respectively) and at the following budburst in 2012 (2012/05/02 or 2012/05/09 depending on the trees). For each of these dates, we sampled 2011 and 2010 light growth units with a shotgun, two trunk cores containing phloem and xylem (at breast height, i.e. 1.30 m). To assess the quantity of living cells in the xylem, a ca. 8 cm. long stem core was sampled at breast height in March 2011. For ring width and wood density measurements, the trees were cored to the pith in June 2011.

Samples were brought back in a cooler to the laboratory, where they were weighted for fresh weight. They were then immediately placed in a -80°C freezer. The samples destined for carbohydrates analysis were lyophilized for seven days, and weighted for dry weight just after

freeze-drying to estimate humidity content for each organ. The samples destined for protein assessment were kept frozen until analysis.

Wood characteristics and anatomy

Increment cores for living cells assessments were freeze-dried, cut tangentially with a sliding microtome at increasing distances from the cambium (0,1, 4 and 7 cm), and stained with Coomasie blue to reveal living cells. Coomassie blue dyes only the proteins of the cytoplasm. The living cells are therefore dyed blue, while dead cells remain colorless. The resulting slides were photographed under a macroscope (Nikon AZ100, Nikon Instruments, Japan), and the photographs were processed with the ImageJ software (National Institutes of Health, USA). For each slide, the number and total area of parenchyma rays per xylem area were measured in three different fields (2.4 mm in diameter). In each of these fields, three parenchyma rays were chosen for length and width measurements. These measurements were then averaged for each tree.

The increment cores reaching the pith were analyzed by indirect X-ray densitometry (Polge 1966). The resulting X-ray films were scanned at 1000 dpi resolution, with 8 bits depth. The digital images were processed with WinDENDRO. Width and density of ring were measured. The ring width and wood densities were averaged over the last five and ten years. Additionally, we computed averages for the 1976-2003 and 2003-2010 periods, 1976 and 2003 corresponding to the last major drought events.

Stem maintenance respiration

Stem maintenance respiration was measured during the 2011 winter season, at 4 different dates corresponding to 4 different mean temperatures (3, 12, 16 and 19°C). We used an Infra-Red Gas Analyzer (EGM-4, PP Systems, USA), and adapted a PP systems soil respiration

chamber to trunk respiration measurements. A polyvinyl chloride base was attached on each tree trunk, oriented South-East (where all the trunk were flat enough to achieve air tightness). The soil respiration chamber was then placed on the base, sealed with Terostat-VII (Teroson, Henkel, Germany), and held in place with a tie-down ratchet strap. Each measurement lasted three minutes, during which the accumulation of CO_2 in the chamber was monitored every minute as an elevation of CO_2 concentration. Temperature was noted at the beginning of each measurement. Between three and five measurements per tree were performed on each date.

Respiratory flux R (µmol m^{-2} s^{-1}) was computed as follows:

$$R = \frac{[CO_2] * \frac{V_c}{V_m}}{S * t}$$

Where the CO_2 concentration in the chamber is expressed in ppm, V_c (volume of the chamber) in m^3 V_m (air molar volume) in m^3 mol^{-1}, S (trunk surface covered by the chamber) in m^2, and t (duration of the measurement) in s.

From the respiratory flux data at different temperature, the Q_{10} (the factor by which the respiratory flux is multiplied for a 10 °C temperature elevation) was determined using the following relationship:

$$R_T = R_{15}.Q_{10}^{\frac{T-15}{10}}$$

Where R_T and R_{15} are the respiratory flux at temperature T (in °C) and at 15°C respectively, expressed in the same units as above.

Non structural carbohydrates

Soluble sugars and starch were extracted from 10 mg (± 0.5 mg) of finely grounded dry matter of 2011 and 2010 growth units, phloem, 0.5 outermost cm of xylem. Grounding was achieved with a ball mill (MM 301, Retsch, Germany) using 5 mm balls and shaking at 30 Hz three times for 2 min. Soluble sugars were extracted twice with 1 mL of boiling 80% ethanol.

These 2 mL were pooled and centrifuged. The supernatants and the pellets containing starch were dried in a centrifugal vacuum concentrator to remove ethanol (Centrivap, Labconco, USA). Soluble sugars and the pellets containing starch were then rehydrated with 0.02 N NaOH. Glucose, fructose and sucrose were assessed enzymatically with the method described in (Barbaroux et al. 2003; Brummer and Cui 2005). Starch was quantified as glucose equivalents, with the enzymatic method described in the same two articles as for soluble sugars.

Total non-structural nitrogen

Soluble proteins were assessed in the 2011 and 2010 growth units, the phloem, the 1.5 outermost cm of xylem with an adaptation of a protocol described in (Bahrman et al. 1997). 400 mg of fresh matter were grounded in liquid nitrogen. The proteins were then precipitated with 10 mL of precipitation solution (acetone, 10% TCA and 0.07% β-mercapto-ethanol). After a night at -20°C, the extracts were centrifuged (15 min, -4°C, 12000 xg), and the supernatants eliminated. The remaining pellets were rinsed with 10mL 0.07% β-mercapto-ethanol in acetone, incubated 1 hour at -20°C, centrifuged (30 min, 4°C, 12000 xg), and the supernatants were again eliminated. The pellets were dried in a centrifugal vacuum concentrator (Centrivap, Labconco, USA). 10 to 20 µL by mg of pellet of a solubilization solution (7M urea, 2M thiourea, 100mM DTT, 4% CHAPS, 0.4% Tritonx100 in water) were added. After a 30 min sonication, the tubes were centrifuged (30 min, 15°C, 12000 xg), and the supernatants were transferred in other tubes.

Proteins were assessed in these supernatants by the Bradford method (Bradford 1976). Bradford reagent (BioRad Protein Assay, BioRad, USA) was diluted four times, and 3.5 mL of the diluted dye reagent were added to 10 µL of the protein extract mixed with 10 µL of HCl 0.1 N and 80 µL of distilled water. After 15 min in the dark, absorbance was read at 595

nm with a spectrophotometer (UVIKON 938, NorthStar, UK). Protein concentration was calculated using solutions of increasing ovalbumine concentrations as a standard.

Frost hardiness

Branches 30 to 50 mm long were sampled during the 2012-2013 winter (2013/01/29) with a shotgun for frost hardiness quantification. Three replicates were made for each tree. The electrolyte leakage test was used to estimate the semi-lethal temperatures of branch segments, as described in Charrier & Améglio (2011). The test is based on the fact that damage to cellular membranes results in electrolyte leakage from the cells. Briefly, 5-cm-long branch segments were submitted to four sub-zero temperatures (-5°C, -10°C, -20°C and -30°C). Additionally, one control segment was kept at +5°C and a lethal control segment was frozen at -80°C. Electrolyte leakage was quantified by measuring the conductivity of a solution in which the branch segments were shaken (C_1). This conductivity was compared to the conductivity of this same solution after being autoclaved at +120°C for 30 minutes (C_2). The relative electrolyte leakage (REL) was expressed as:

$$REL = \frac{C_1}{C_2} * 100$$

Statistical analysis

Data were analyzed with R software (http://www.r-project.org/).

Q_{10} and R_{15} were determined by fitting a non-linear model to our data with the least squares method, using the nls function in r. Two methods were used: either one model was fitted for each health status, or one model was fitted for each tree. Models obtained with the first method were fitted on more data points than those form the second one, making the estimates more robust. However, the second method enabled us to test for a health status effect one Q_{10} and R_{15}.

Semi-lethal temperatures (LT50) were determined by fitting one logistic model per tree to the data from the electrolyte leakage test, as described in Charrier & Améglio (2011).

The effect of health status onQ10, R15, LT50 and reserve concentrations was tested with Student's t-tests. To assess a potential difference of reserve dynamics between healthy and declining trees, three synthetic variables were calculated: the difference between the winter and yellowing concentrations (reserve use at the beginning of the leafless season), the difference between the budburst and winter concentrations (reserve use during the second part of the leafless season), and the difference between the yellowing and budburst concentrations (reserve use during the whole leafless season). The effect of health status on these three variables was tested with Student's t-tests. Physiologically relevant correlations between variables were tested with Pearson's method. Tests were considered conclusive at $P<0.05$.

Results

Living tissue and structural characteristics in the xylem

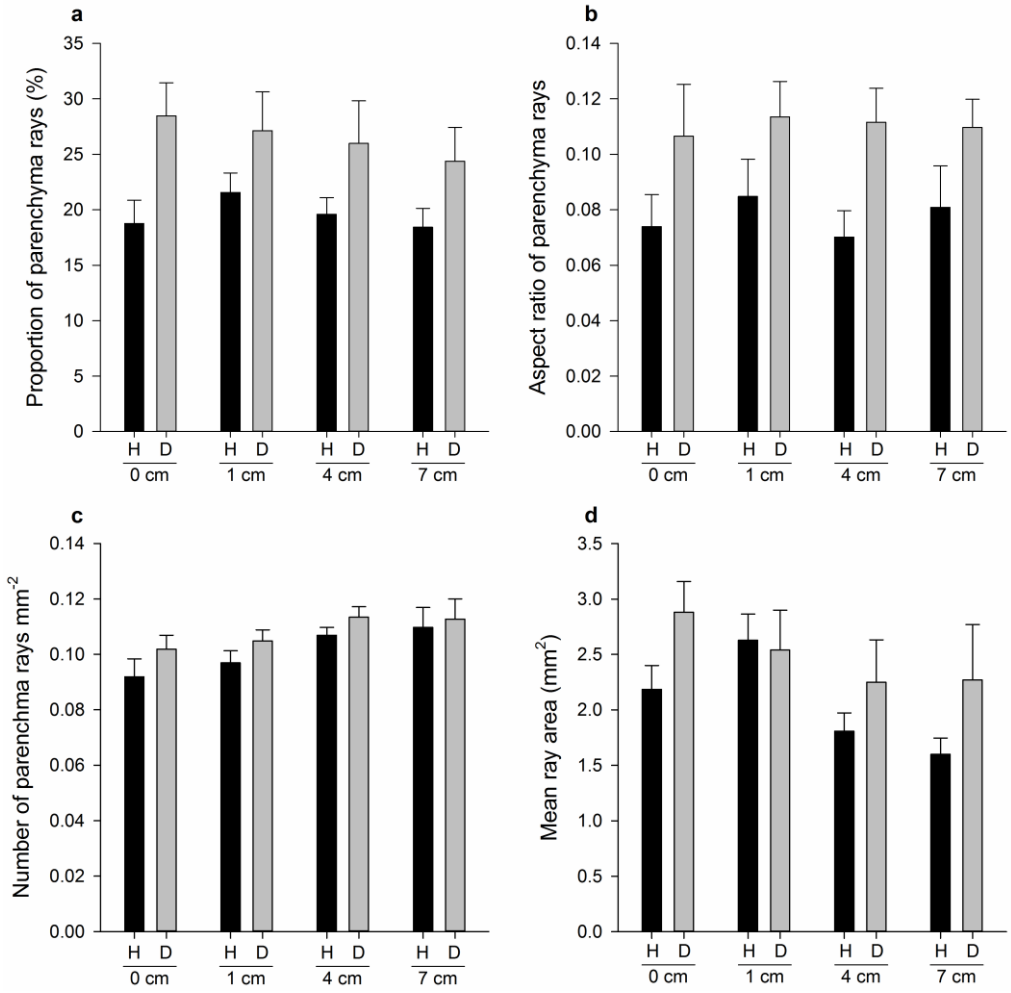

Figure III.1 Evolution of the proportion of parenchyma rays in percentage of wood surface (a), the aspect ratio of parenchyma rays on tangential cut (b), the number of parenchyma ray per area (c), and the mean area of one parenchyma ray (d) with increasing distances from the cambium (0 cm, 1 cm, 4 cm and 7cm) in healthy (H, black) and declining (D, grey) beech trees growing in the Fontainebleau state forest (France). Error bars represent SE (n=5).

Radial parenchyma represented a significantly lower (P<0.05) proportion of wood surface on tangential section in healthy trees (19.6±1.7 %) compared to declining trees (25.9±3.0 % Figure III.1a). On average, this proportion did not vary with increasing distances from the cambium (Figure III.1a). However, the number of rays per area significantly increased with increasing distances from the cambium (P<0.05, Figure III.1c), while the mean surface per ray decreased from 2.5±0.2 mm^2 at the cambium to 1.9±0.3 mm^2 7 cm away from

the cambium (P<0.05) in both health statuses (Figure III.1d). The shape of radial parenchyma rays differed between healthy and declining trees. Healthy trees had long and thin rays (Figure III.2a), whereas the parenchyma rays in declining trees were short and wide (Figure III.2b). The aspect ratio of radial parenchyma rays (mean ray width/ray length) was therefore higher in declining trees (P<0.05, Figure III.1b). The aspect ratio of radial parenchyma rays was not affected by the distance of the cut from the cambium (Figure III.1b). Neither ray length nor ray width was significantly different between healthy and declining trees.

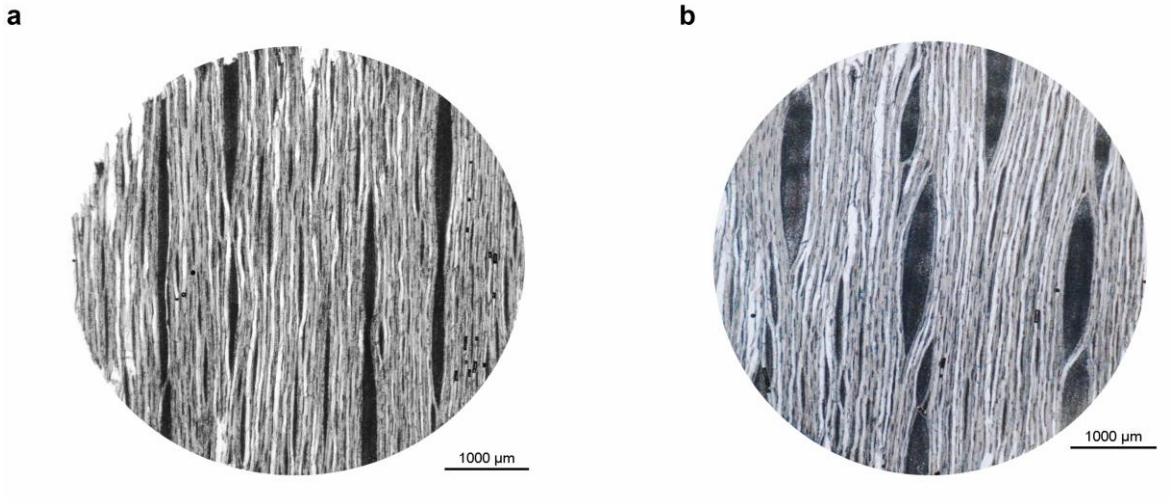

Figure III.2 Examples of tangential section used for the measurement of parenchyma rays in healthy (a) and declining (b) beech trees.

Concerning structural wood features, the mean ring width of declining trees was significantly lower than the one of healthy trees, whatever the chosen period (Table III.1). The mean ring densities were however similar for healthy and declining trees for all the studied time intervals (Table III.1).

	Period	**Healthy trees**	**Declining trees**
Ring width (mm)	Last 5 years	1.64 ± 0.28	0.83 ± 0.24
	Last 10 years	1.64 ± 0.31	0.86 ± 0.21
	2003-2010	1.57 ± 0.30	0.82 ± 0.22
	1976-2003	2.47 ± 0.47	1.40 ± 0.35
Average ring density	Last 5 years	0.60 ± 0.02	0.56 ± 0.0.2
	Last 10 years	0.61 ± 0.02	0.57 ± 0.02
	2003-2010	0.59 ± 0.02	0.56 ± 0.02
	1976-2003	0.66 ± 0.02	0.64 ± 0.02

Table III.1 Mean (± standard errors) ring widths and densities of healthy and declining beech trees growing in the Fontainebleau state forest (France).

Maintenance respiration and frost hardiness

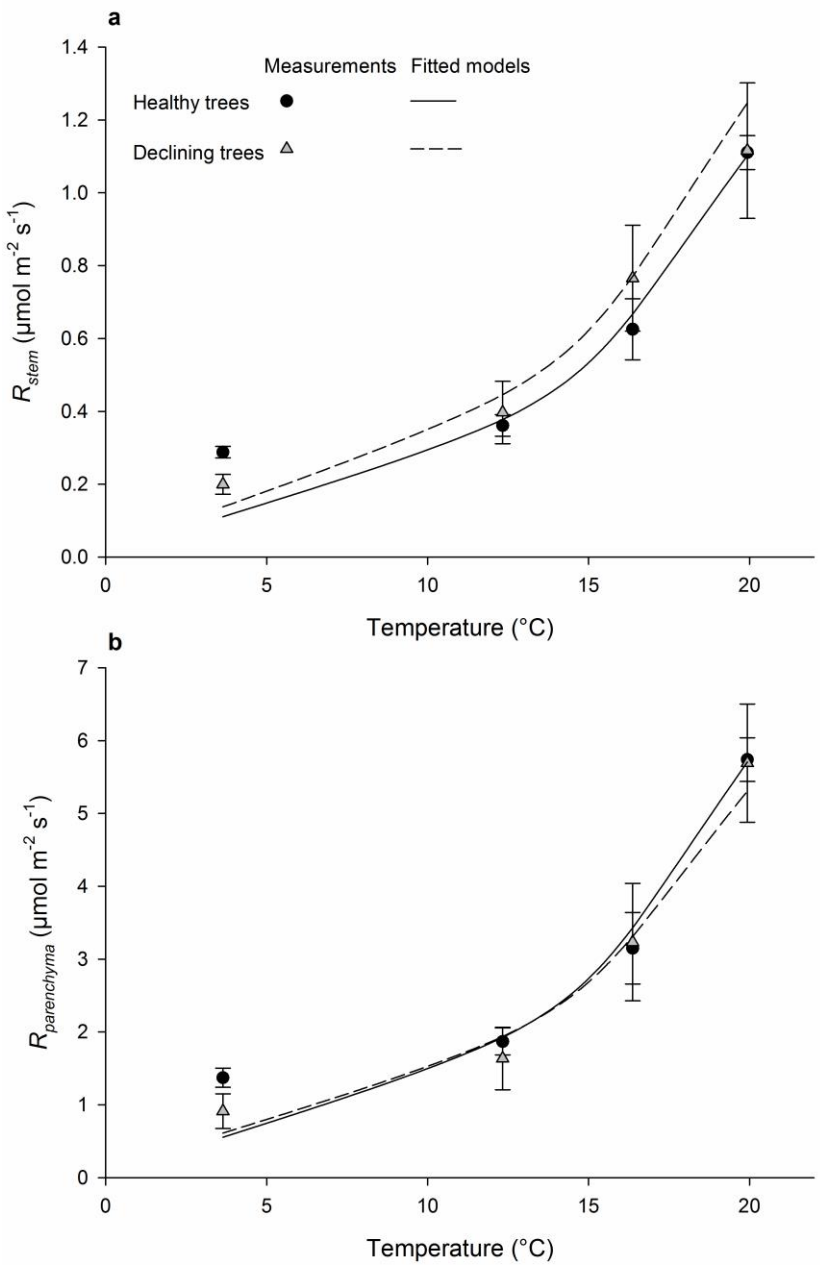

Figure III.3 Response of the maintenance respiration (R, µmol m-2 s-1) of healthy (black circles) and declining (grey triangles) beech trees to temperature in Fontainebleau state forest (France), either expressed per stem area (a), or per living cell area (b). Models fitted over the respiration data are represented by a solid line (healthy trees) and a dashed line (declining trees). Error bars represent SE (n=5).

Stem maintenance respiration was expressed either per stem area, or per living cell area. In both cases, it was similar for healthy and declining trees at each measurement date (Figure III.3). The respiration rate per stem area normalized at 15°C (R_{15}), as estimated by fitting one

model per tree, was 0.53±0.04 µmol CO_2 m^{-2} s^{-1} for healthy trees and 0.48±0.15 µmol CO_2 m^{-2} s^{-1} for declining trees, which was not significantly different. The factor by which the respiratory rate increased for a 10°C increase in temperature (Q_{10}) was similar for healthy (4.60±0.49) and declining trees (3.55±0.46) when expressed per stem area. Similar estimations of Q_{10} and R_{15} were obtained when respiration rates were expressed per living cell area. Fitting one "mean" model per health status resulted in similar estimations of Q_{10} and R_{15} (Data not shown).

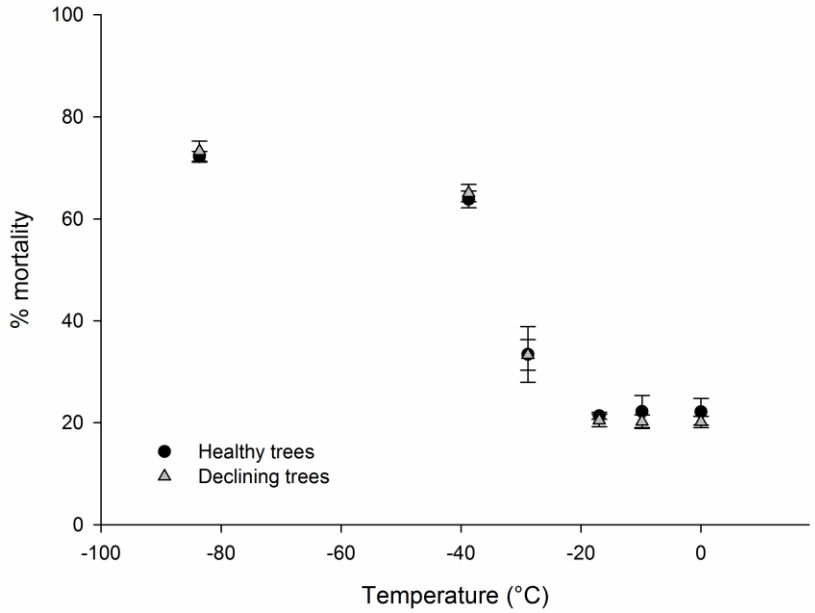

Figure III.4 Temperature response of the percentage of dead cells as assessed by the electrolyte leakage test in healthy (black circles) and declining (grey triangles) beech trees. Error bars represent SE (n=5).

Frost hardiness was quantified by the temperature causing the death of 50% of living cells (LT_{50}), as measured by the electrolyte leakage test. Health status had no significant effect on LT_{50} values (Figure III.4): LT_{50} were 32.8±1.2°C and -32.6±0.7°C for healthy and declining trees respectively. The slopes of the logistic curve were also similar for the two health statuses (Data not shown).

C reserves

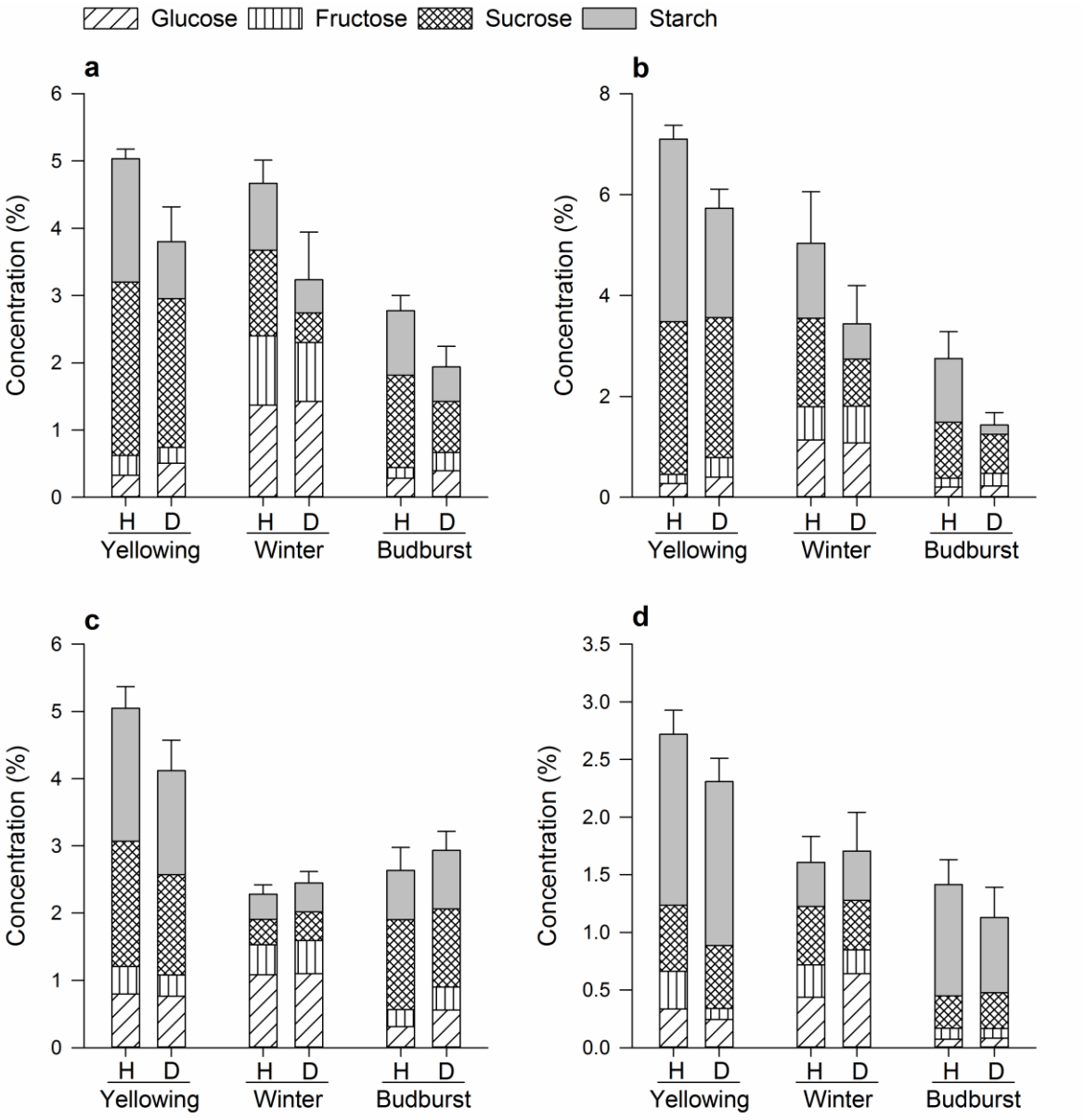

Figure III.5 Concentrations of carbon reserves in healthy (H) and declining (D) beech trees in the Fontainebleau state forest (France), at yellowing and in the middle of winter in 2011, and at the following budburst in 2012 in the 2011 branches (a), the 2010 branches (b), the phloem (c), the xylem (d). Carbon reserves are divided in glucose (slanted stripes), fructose (straight stripes), sucrose (squares) and starch (solid grey). The error bars represent SE.

TNSC concentrations differed between healthy and declining trees only in the 2010 branches at yellowing (P<0.05), and were otherwise similar (Figure III.5). In the phloem, TNSC concentrations decreased more in healthy trees (75.2±11.4 %) than in declining trees

(51.6±4.9 %) between leaf yellowing and the following budburst (P<0.05). In the other organs studied, TNSC dynamics were similar in healthy and declining trees (Figure III.5). Starch concentrations were lower in declining trees than in healthy trees at yellowing in the 2011 and 2010 branches, and at the following budburst in the 2010 branches (Figure III.5 a and b). Health status had no significant effect on starch concentrations in the other organs studied (Figure III.5). Starch concentrations decreased steadily during the whole leafless season in 2011 and 2010 branches. In the stem phloem and xylem, starch concentrations decreased between yellowing and the middle of winter, and increased thereafter (Figure III.5 c and d). Total soluble sugar concentrations were similar for healthy and declining trees in all the organs studied, whatever the date (Figure III.5). When separated between glucose, fructose and sucrose, no consistent concentration difference was found between healthy and declining trees. The soluble sugars dynamics were mainly similar between healthy and declining trees. Soluble sugar concentrations increased between yellowing and the middle of winter, and decreased between winter and the following budburst (Figure III.5). Only one exception was found for glucose in the stem xylem: between yellowing and the middle of winter, glucose concentrations increased more in declining trees than in healthy trees (P<0.05, Figure III.5d).

When starch and TNSC concentrations were divided by the proportion of living cells, no difference was found between healthy and declining trees for starch, whatever the date (Data not shown). For TNSC, however, at yellowing, healthy trees appeared to store more C reserves for a given living cell area than declining trees (Data not shown).

N reserves

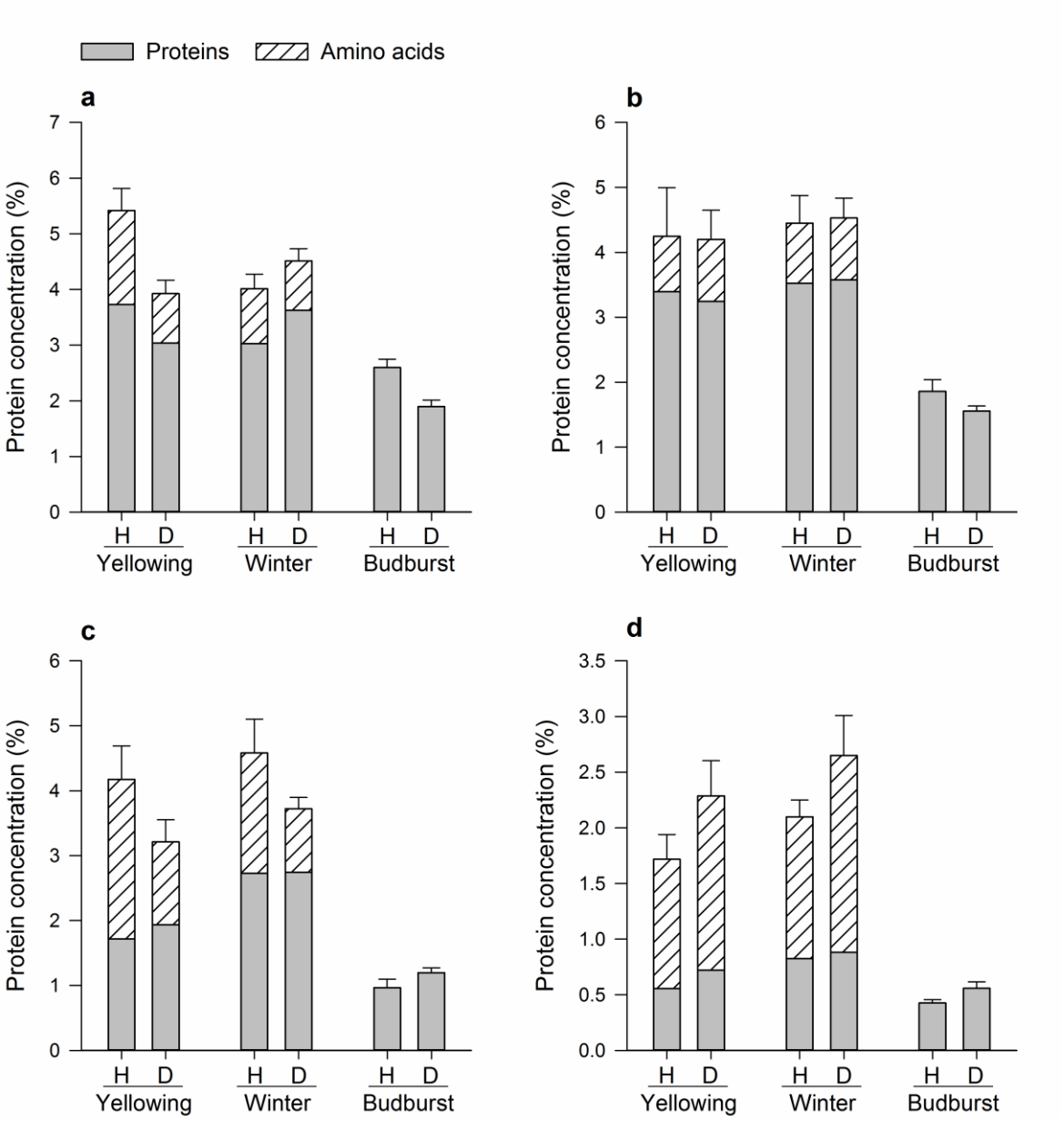

Figure III.6 Concentrations of nitrogen reserves in healthy (H) and declining (D) beech trees in the Fontainebleau state forest (France), at yellowing and in the middle of winter in 2011, and at the following budburst in 2012 in the 2011 branches (a), the 2010 branches (b), the phloem (c), the xylem (d). Nitrogen reserves are divided in soluble proteins (solid grey) and amino acids (slanted stripes). The error bars represent SE.

Whatever the sampling date, TNSN concentrations were mainly similar for healthy and declining trees. The only differences were found in the 2011 branches at yellowing, where healthy trees had lower TNSN concentrations (P<0.05, Figure III.6a). For soluble protein concentrations, healthy trees has greater soluble protein concentrations than declining trees in

the 2011 branches at budburst (P<0.05, Figure III.6a). In the stem xylem, healthy trees had lower soluble protein concentrations than declining trees at yellowing (P<0.01, Figure III.6d). In the other organs studied, soluble protein concentrations were similar in healthy and declining trees whatever the date (Figure III.6 b, c and e). Soluble protein concentrations decreased in a greater extent between winter and budburst in declining trees than in healthy trees in the 2011 branches (P<0.05, Figure III.6a). In the other organs studied, soluble protein concentration dynamics were similar for healthy and declining trees (Figure III.6). Amino acid concentrations were significantly different for healthy and declining tree only in the phloem in the middle of winter (P<0.05). These concentration differences were a consequence of a greater increase of amino acid concentrations in healthy trees than in declining trees between yellowing and the middle of winter (P<0.05, Figure III.6 c and e).

Relationships between structural and functional features

Physiologically relevant correlations between the studied variables were tested. Frost hardiness was not correlated with amino acid concentrations, soluble sugar concentrations, or soluble sugar and amino acid concentrations cumulated. R_{15} was not correlated with C nor N reserve concentrations and dynamics, nor was it correlated with the proportion of radial parenchyma in wood or the aspect ratio of parenchyma rays. The aspect ratio of the parenchyma rays was however positively correlated with the TNSN concentration in the xylem at yellowing (P<0.05, R^2=0.42, Data not shown). The proportion of parenchyma rays in the xylem on tangential cut was significantly correlated with the average density of the xylem of the last 5 years (P<0.01, R^2=0.67, Figure III.7a), of the last 10 years (P<0.01, R^2=0.62, Data not shown), and with the average density of the xylem of the 2003-2010 period (P<0.01, R^2=0.61). However, this correlation was not significant over the 1976-2003 period (Data not shown). The correlation between the aspect ratio of parenchyma rays on tangential section

and the average ring width of the last 5 years was also found significant (P<0.05, R^2= 0.49, Figure III.7b). However, the aspect ratio of the parenchyma rays was not correlated with the average ring width of the last 10 years, or of the 2003-2010 and 1976-2003 periods (Data not

shown).

Discussion

The purpose of this study was to examine the winter stem ecophysiological processes

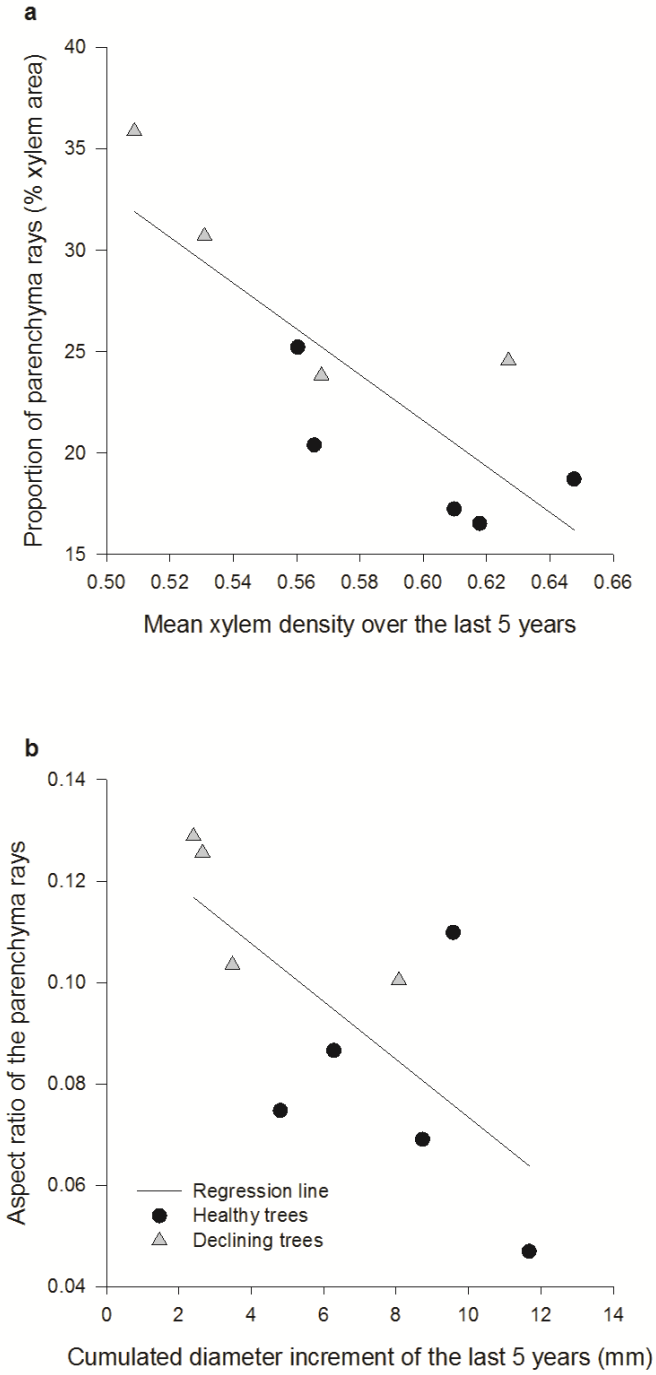

Figure III.7 Relationship between the proportion of parenchyma rays and the average wood density of the last 5 years (a), and between the aspect ratio of parenchyma rays and the average diameter increment of the last five years (b) in healthy (black circles) and declining (grey triangles) beech trees.

occurring in the aboveground woody compartment of mature healthy and declining beech trees and to relate them to potential changes in the xylem anatomy. These processes include frost hardening and tissue maintenance, both central to the survival of parenchyma cells (the living part of the xylem) during winter, and thus to the resuming of growth in spring.

Abundance of radial parenchyma

Radial parenchyma represented on average 22.8±2.0 % of the wood surface on tangential section. In the literature, the proportion of parenchyma rays has been reported to be around 20% in beech wood (Burgert and Eckstein 2001; Ceschia et al. 2002). The xylem of declining trees contained a higher proportion of radial parenchyma than the xylem of healthy trees (Figure III.1a), which contradicts our initial hypothesis that declining trees would have less living tissue in the xylem than healthy trees. In the literature, tree vigour and the proportion of parenchyma in the xylem are usually positively correlated (Gartner et al. 2000; Fonti et al. 2015). However, Fonti et al. (2015) proposed an alternative hypothesis: as under stressful conditions, active storage could decrease the risk of C starvation (Wiley and Helliker 2012), trees growing in stressful conditions could show an increased storage capacity, leading to a greater proportion of parenchyma rays in the xylem (Fonti et al. 2015).

Proportion of parenchyma in the xylem in relation to storage

The increased maximum storage capacity, as approximated by the proportion of parenchyma rays in the xylem, was not associated with consistently increased C or N reserve concentrations in the trees studied (Figure III.5). Conversely, declining trees tended to have lower TNSC concentrations, and similar TNSN concentrations than healthy trees (Figure III.5 and 6). The observed C reserve concentrations and winter dynamics were consistent with values and patterns reported for beech in the literature (Barbaroux and Bréda 2002; El Zein et

al. 2011b; Gérard and Bréda 2012; Scartazza et al. 2013). C reserve concentrations were maximum at yellowing, and decreased throughout winter in the branches and xylem (Figure III.5 a, b and d). In the phloem, C reserve concentrations decreased during the first part of winter, and increased slightly at the following budburst. This increase is mainly due to an increase in the concentration (Figure III.5c) sucrose, which is the main circulating form of carbohydrates in the phloem (Zimmermann 1957). This increase could therefore be linked to C reserve remobilization processes in order to provide C substrates to the expanding leaves.

At yellowing, when reserve concentrations were maximum, healthy trees stored greater C reserve concentrations by percent of living tissue in the xylem. This suggests that healthy trees store C reserves more efficiently, entailing that they could need a lower proportion of living tissue in the xylem than declining trees to store the same amount of C reserves.

Maintenance costs

The greater proportion of living cells in the xylem of declining trees was however not associated with greater CO_2 efflux (Figure III.3). The measured maintenance respiration rates, expressed by unit of wood surface, were coherent with the range values reported in the stem of beech trees (between 0.2 and 0.5 µmol CO2 m^{-2} s^{-1}, Ceschia et al. 2002, Damesin et al. 2002). The response of maintenance respiration to temperature variations, as estimated by the Q_{10}, was similar in healthy and declining trees (Figure III.3). C losses by maintenance respiration are therefore likely to be similar for healthy and declining trees throughout the winter. Coherent with the similar respiration rates found for healthy and declining trees, TNSC concentrations decreased in similar proportions in healthy and declining trees between yellowing and the following budburst (Figure III.5). To our knowledge, no study has specifically measured maintenance respiration in mature trees during decline. The available data in the literature comes from drought experiments. In Norway spruce, the respiration rate

decreased in response to a drought treatment (Hartmann et al. 2013b). Conversely, Mitchell et al. (2013) showed that the dark respiration rate was not affected by drought until the last 1 -2 weeks before death in two Eucalyptus species. Both studies were carried out on young plants subjected to extreme experimental droughts, and are therefore not directly comparable to our results.

The higher proportion of living cells in the xylem of declining trees did not lead to a lower respiration rate per area of parenchyma. In the literature, higher parenchyma volumes in the xylem can be associated with a decreased respiratory activity when expressed per volume of parenchyma cells (Rodriguez-Calcerrada et al. 2015). The difference of proportion of living cells in the xylem of healthy and declining trees (around 5%) might not be sufficient to induce significant changes in the respiration rates at the intra-specific level. At the inter-specific level however; small differences of proportion of parenchyma in the xylem of three angiosperm tree species significantly altered the intensity of the respiration (Spicer and Holbrook 2007). Moreover, the stem maintenance respiration rate is usually correlated with the N content of the wood (Ryan 1995; Ceschia et al. 2002; Rodriguez-Calcerrada et al. 2015). As declining trees tended to have greater N reserve concentrations than healthy trees in the stem xylem (Figure III.6d), healthy and declining trees had similar N reserve concentrations per percentage of living cells (Data not shown). Given that N reserve concentrations are usually correlated with the total N content of the wood (Gomez and Faurobert 2002), the N content of living cells is probably similar in healthy and declining trees, which is coherent with the similar respiration rates when expressed by surface of living cells.

Cold hardiness

C reserves, and more precisely soluble sugar, act during winter both as energy sources and as a protection against frost damages (Charrier and Améglio 2011). Some amino acids are also associated with increased frost hardiness (Janská et al. 2010). C and N reserve concentrations have therefore a potential impact on the cold hardiness of trees (Charrier et al. 2013). Healthy and declining trees had similar cold hardiness (Figure III.4), which is coherent with the generally similar soluble sugar and amino acid concentrations in both health statuses. Moreover, the maximum frost hardiness does not seem to be influenced by the environmental conditions at the intra-specific level (Bower and Aitken 2006; Charrier et al. 2011), which seems to hold true even in case of tree decline. The measured semi-lethal temperatures were in ranges with those reported for *Fagus sylvatica* (Charrier et al. 2013) and *Fagus crenata* (Gansert et al. 1999). The living cells of the xylem of healthy and declining trees are thus likely to have similar survival rates to frost events.

Functional implication of the shape of parenchyma rays

In winter, the survival of perennial organs relies both on the frost resistance of the living cells and on the resistance to freeze-induced embolism in the xylem vessels (Pratt et al. 2005). The latter was not specifically assessed in this study. In beech, the loss of conductivity usually increases progressively during winter (Borghetti et al. 1993; Cochard et al. 2001). Part of the restoration of the hydraulic conductivity is an active mechanism, relying on a positive pressure at the base of the stem (Cochard et al. 2001). This active mechanism could be dependent on the functional connexion between the living cells of parenchyma rays and the xylem vessels.

The functional connexion between the living cells of the parenchyma and the conducting elements of the xylem might be in part determined by the characteristics of the parenchyma rays (Fonti et al. 2015). The shape of the radial parenchyma rays was significantly affected by

the health status of the trees: the parenchyma rays of healthy trees appeared as long and thin, whereas those of declining trees had a rounder shape (Figures III.1b and III.2). This difference in shape could have functional implications: as discussed in Fonti et al. (2015), parenchyma in the xylem works in connexion with the conductive tissues (xylem and phloem). The rounder shape of parenchyma rays in declining trees could mean that a lower proportion of cells in the rays are in direct contact with the conductive tissues. The connexion between parenchyma and the conductive tissues might therefore be altered in declining trees, hindering the ability of the parenchyma to play its role in the storage and transport of water and reserves (Fonti et al. 2015).

The characteristics of parenchyma rays are often linked to the structure of the xylem (Fonti et al. 2015). The correlations between the proportion of parenchyma and wood density (Figure III.7a) and between the aspect ratio of parenchyma rays and ring widths (Figure III.7b) confirm the existence of such a link in beech trees. Ring width and wood density are, like other wood structural characteristics, in part determined by processes occurring during the growing season (Bouriaud et al. 2004; Galván et al. 2014). Processes occurring during the growing season could therefore potentially impact tree functioning during winter and the following years, not only via the C and N allocation processes, via the permanence of xylem anatomical traits.

Conclusion

This study provides new insights into the winter ecophysiology of the aboveground woody compartment in a context of tree decline. Under temperate climates, the ability to survive winter is crucial for the long-term survival of trees. Healthy and declining trees had very similar ecophysiological characteristics (respiration rate, frost hardening, C and N reserve concentrations and dynamics). However, the quantity of living cells in the xylem was

increased in declining trees. As a result, the storage of carbon reserves in declining trees might require more living cells than in healthy trees for similar reserve concentrations, making it less efficient. Moreover, in declining trees, the functional connexion between parenchyma rays and the conducting elements of the xylem might be altered because of the different shape of parenchyma rays in healthy and declining trees. These differences in parenchyma abundance and structure is associated with other characteristics of the xylem (ring width and wood density), suggesting a role of structural constraints in the formation of parenchyma rays. A more thorough study of the functional anatomy of the xylem of healthy and declining trees could clarify the link between the shape of the parenchyma rays and their function. Moreover, the association of wood anatomy and ecophysiological data might help to develop a better understanding of the fine functional mechanisms underlying tree decline and mortality. Furthermore, the study of functional anatomical features in tree rings could offer a retrospective insight of the long-term history of tree decline.

References

Anderegg WRL, Anderegg LDL, Berry JA, Field C (2014) Loss of whole-tree hydraulic conductance during severe drought and multi-year forest die-off. Oecologia 175:11–23.

Bahrman N, Plomion C, Petit RJ, Kremer A (1997) Contribution of two-dimensional electrophoresis of proteins to maritime pine genetics. Ann For Sci 54:225–236.

Barbaroux C, Bréda N (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. Tree Physiol 22:1201–1210.

Barbaroux C, Bréda N, Dufrêne É (2003) Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (Quercus petraea and Fagus sylvatica). New Phytol 157:605–615.

Borghetti M, Leonardi S, Raschi A, et al. (1993) Ecotypic Variation of Xylem Embolism, Phenological Traits, Growth Parameters and Allozyme Characteristics in Fagus sylvatica. Funct Ecol 7:713–720.

Bouriaud O, Bréda N, Le Moguédec G, Nepveu G (2004) Modelling variability of wood density in beech as affected by ring age, radial growth and climate. Trees - Struct Funct 18:264–276.

Bower AD, Aitken SN (2006) Geographic and seasonal variation in cold hardiness of whitebark pine. Can J For Res 36:1842–1850.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Bréda N, Badeau V (2008) Forest tree responses to extreme drought and some biotic events: Towards a selection according to hazard tolerance? Comptes Rendus Geosci 340:651–662.

Brummer Y, Cui SW (2005) Understanding Carbohydrate Analysis. In: Food Carbohydrates : Chemistry, Physical properties and Applications. Taylor and Francis group, pp 67–104.

Burgert I, Eckstein D (2001) The tensile strength of isolated wood rays of beech (Fagus sylvatica L.) and its significance for the biomechanics of living trees. Trees - Struct Funct 15:168–170.

Ceschia É, Damesin C, Lebaube S, Pontailler JY, Dufrêne É (2002) Spatial and seasonal variations in stem respiration of beech trees (Fagus sylvatica). Ann For Sci 59:801–812.

Chapin SF, Schultze E-D, Mooney HA (1990) The Ecology and Economics of Storage in Plants. Annu Rev Ecol Syst 21:423–447.

Charrier G, Améglio T (2011) The timing of leaf fall affects cold acclimation by interactions with air temperature through water and carbohydrate contents. Environ Exp Bot 72:351–357.

Charrier G, Bonhomme M, Lacointe A, Améglio T (2011) Are budburst dates, dormancy and cold acclimation in walnut trees (Juglans regia L.) under mainly genotypic or environmental control? Int J Biometeorol 55:763–774.

Charrier G, Cochard H, Améglio T (2013) Evaluation of the impact of frost resistances on potential altitudinal limit of trees. Tree Physiol 33:891–902.

Cochard H, Lemoine D, Améglio T, Granier A (2001) Mechanisms of xylem recovery from winter embolism in Fagus sylvatica. Tree Physiol 21:27–33.

Damesin C, Ceschia É, Le Goff N, Ottorini J-M, Dufrêne É (2002) Stem and branch respiration of beech: from tree measurements to estimations at the stand level. New Phytol 153:159–172.

Fonti P, Tabakova M, Kirdyanov A, Bryukhanova M, von Arx G (2015) Variability of ray anatomy of Larix gmelinii along a forest productivity gradient in Siberia. Trees - Struct Funct:1–11.

Galván JD, Camarero JJ, Gutiérrez E (2014) Seeing the trees for the forest: drivers of individual growth responses to climate in Pinus uncinata mountain forests. J Ecol:1244-1257.

Gansert D, Backes K, Kakubari Y (1999) Altitudinal and seasonal variation of frost resistance of Fagus crenata and Betula ermanii along the Pacific slope of Mt. Fuji, Japan. J Ecol 87:382–390.

Gartner BL, Baker DC, Spicer R (2000) Distribution and vitality of xylem rays in relation to tree leaf area in douglas fir. IAWA J 21:389–401.

Gérard B, Bréda N (2012) Radial distribution of carbohydrate reserves in the trunk of declining European beech trees (Fagus sylvatica L.). Ann For Sci 69:1–8.

Gomez L, Faurobert M (2002) Contribution of vegetative storage proteins to seasonal nitrogen variations in the young shoots of peach trees (Prunus persica L. Batsch). J Exp Bot 53:2431–2439.

Hartmann H, Ziegler W, Trumbore S (2013) Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy. Funct Ecol 27:413–427.

Intergovernmental Panel on Climate Change (2014) Climate Change 2014: Synthesis Report.

Janská A, Maršík P, Zelenková S, Ovesná J (2010) Cold stress and acclimation – what is important for metabolic adjustment? Plant Biol 12:395–405.

Kreyling J (2010) Winter climate change: a critical factor for temperate vegetation performance. Ecology 91:1939–1948.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manage 259:698–709.

Manion PD (1981) Tree disease concepts. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.

McDowell NG (2011) Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. Plant Physiol 155:1051–1059. doi: 10.1104/pp.110.170704

McDowell NG, Pockman WT, Allen CD, Breshears DD, Cobb NS, Kolb T, Plaut JA, Sperry JS, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739.

Mitchell PJM, O'Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytol 197:862–872.

Montwé D, Spiecker H, Hamann A (2014) An experimentally controlled extreme drought in a Norway spruce forest reveals fast hydraulic response and subsequent recovery of growth rates. Trees - Struct Funct 28:891–900.

Moulinier J, Lorenzetti F, Bergeron Y (2014) Growth and mortality of trembling aspen (Populus tremuloides) in response to artificial defoliation. Acta Oecologica 55:104–112.

Nageleisen L-M, Goudet M (2011) Manuel de Notation des dommages forestiers (symptômes, causes, état des cimes). Paris.

Ögren E (2000) Maintenance respiration correlates with sugar but not nitrogen concentration in dormant plants. Physiol Plant 108:295–299.

Ögren E (2001) Effects of climatic warming on cold hardiness of some northern woody plants assessed from simulation experiments. Physiol Plant 112:71–77.

Oliet JA, Puértolas J, Planelles R, Jacobs DF (2013) Nutrient loading of forest tree seedlings to promote stress resistance and field performance: A Mediterranean perspective. New For 44:649–669.

Penning de Vries FWT (1975) The Cost of Maintenance Processes in Plant Cells. Ann Bot 39:77–92.

Polge H (1966) Établissement des courbes de variation de la densité du bois par exploration densitométrique de radiographies d'échantillons prélevés à la tarière sur des arbres vivants : applications dans les domaines Technologique et Physiologique. Ann des Sci For 23:1–206.

Pratt RB, Ewers FW, Lawson MC, Jacobsen AL, Brediger MM, Davis SD (2005) Mechanisms for tolerating freeze–thaw stress of two evergreen chaparral species: Rhus ovata and Malosma laurina (Anacardiaceae). Am J Bot 92:1102–1113.

Rodriguez-Calcerrada J, Lopez R, Salomon R, et al. (2015) Stem CO2 efflux in six co-occurring tree species: underlying factors and ecological implications. Plant Cell Environ 38:1104–1115. doi: 10.1111/pce.12463

Ryan MG (1990) Growth and maintenance respiration in stems of Pinuscontorta and Piceaengelmannii. Can J For Res 20:48–57.

Ryan MG (1995) Foliar Maintenance Respiration of Sub-Alpine and Boreal Trees and Shrubs in Relation to Nitrogen-Content. Plant Cell Environ 18:765–772.

Saffell BJ, Meinzer FC, Woodruff DR, Shaw DC, Voelker SL, Lachenbruch B, Falk K (2014) Seasonal carbohydrate dynamics and growth in Douglas-fir trees experiencing chronic, fungal-mediated reduction in functional leaf area. Tree Physiol 34:218–228.

Sakai A (1962) Studies on the Frost-Hardiness of Woody Plants I. The causal relation between sugar content and frost-hardiness. Contrib from Inst Low Temp Sci

Sala A, Piper FI, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol 186:274–281.

Scartazza A, Moscatello S, Matteucci G, Battistelli A, Brugnoli E (2013) Seasonal and inter-annual dynamics of growth, non-structural carbohydrates and C stable isotopes in a Mediterranean beech forest. Tree Physiol 33:730–742.

Spicer R (2014) Symplasmic networks in secondary vascular tissues: parenchyma distribution and activity supporting long-distance transport. J Exp Bot 65:1829–1848.

Spicer R, Holbrook MN (2007) Parenchyma cell respiration and survival in secondary xylem: Does mlanetabolic activity decline with cell age? Plant, Cell Environ 30:934–943.

Tromp J (1983) Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. Plant Soil 71:401–413.

Wiley E, Helliker B (2012) A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. New Phytol 195:285–289.

Zang U, Goisser M, Grams TEE, Häberle K-H, Matyssek R, Matzner E, Borken W (2014) Fate of recently fixed carbon in European beech (Fagus sylvatica) saplings during drought and subsequent recovery. Tree Physiol 34:29–38.

El Zein R, Maillard P, Bréda N, Marchand J, Montpied P, Gérant D (2011) Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. Tree Physiol 31:843–854.

Zimmermann MH (1957) Translocation of Organic Substances in Trees. I. The Nature of the Sugars in the Sieve Tube Exudate of Trees. Plant Physiol 32:288–291.

IV. Soil type and root growth pattern differences, but similar rhizodeposition in a case of beech (*Fagus sylvatica* L.) decline

Soil type and root growth pattern differences, but similar rhizodeposition in a case of beech (*Fagus sylvatica* L.) decline

ALICE DELAPORTE [123], AUGUSTO ZANELLA[4], CLAIRE DAMESIN [123], STEPHANE BAZOT[123]

1. Université Paris-Sud, UMR 8079, Laboratoire Ecologie Systématique et Evolution, Orsay, F-91405, Orsay, France

2. CNRS, UMR 8079, Laboratoire Ecologie Systématique et Evolution, Orsay, F-91405, Orsay, France

3. AgroParisTech, UMR 8079, Laboratoire Ecologie Systématique et Evolution, F-75231 Paris, France

4. University of Padua, Department of Land, Environment, Agriculture and Forestry, Viale dell'Università 16, 35020 Legnaro, Italy

Introduction

Tree decline is a complex phenomenon, with often several biotic and abiotic causes interacting with one another (Manion 1981). Some of these biotic and abiotic factors, for example droughts and/or pest-related damages, might be enhanced in temperate regions as a result of global change (Lindner et al. 2010; Intergovernmental Panel on Climate Change 2014). The effect of global change on the frequency and intensity of future tree decline and mortality events is however still uncertain (Allen et al. 2010). To better predict the impact of global change on forests, it is therefore necessary to develop a fine understanding of the functional mechanisms underlying tree decline and mortality (Bréda and Badeau 2008). Recent research on the ecophysiological responses of trees to moderate or severe droughts has shown that carbon (C) and/or water transport and/or storage could be altered during these events (McDowell et al. 2008, Sala et al. 2010, McDowell 2011, among others). So far, most of the studies have focused mainly on processes occurring in the plant itself. Despite their potential importance in tree functioning (Högberg and Read 2006), processes occurring at the plant-soil interface have seldom been investigated.

The plant-soil interface is commonly designated as the rhizosphere, i.e. the portion of soil under the influence of plant roots. Plant roots significantly modify the soil around them in terms of water and nutrient availability, pH, and microbial activity (Hinsinger et al. 2009) via rhizodeposition, i.e. the release of C by plant roots (Lynch and Whipps 1990). Quantitatively, rhizodeposition is a major component of the C balance of trees: it is estimated that between 40% and 70% of the assimilated C is transferred to the rhizosphere (Grayston et al. 1997). Rhizodeposition strongly stimulates the microbial activity in the vicinity of the roots (Lynch and Whipps 1990). In return, microbes in the rhizosphere can have positive effects on plant productivity by altering nutrient (particularly nitrogen - N) cycles and/or availability (Van Der Heijden et al. 2008). Soil microbial communities can also have negative effects on plant

growth, either directly by acting as pathogens, or indirectly by competing with plants for nutrient acquisition (Van Der Heijden et al. 2008). Plants and rhizospheric micro-organisms tightly interact and highly depend on each other, and therefore plants and soil can be considered as a continuous system (Högberg and Read 2006).

In a context of tree decline, the C balance of trees could be altered (McDowell et al. 2008; Anderegg et al. 2014). More precisely, the photosynthetic C assimilation could be reduced (Anderegg et al. 2014) and/or the C transport by the phloem could be impaired (Sevanto et al. 2014). Girdling experiments have shown that interrupting the C transport by the phloem prevented rhizodeposition, and therefore limited the resource availability of rhizospheric microorganisms (Högberg et al. 2001). Moreover, when the phloem transport is impaired by girdling, the stoechiometry of nutrients in the soil is altered: the inorganic N concentration increases (Högberg et al. 2007; Weintraub et al. 2007), while the organic C and N concentrations decrease (Weintraub et al. 2007; Ekberg et al. 2007; Dannenmann et al. 2009). As rhizodeposition is a major source of labile C for soil microorganisms (Högberg and Read 2006), a diminution of the quantity and/or a modification of the quality of rhizodeposits can result in a decrease of total microbial biomass (Högberg and Högberg 2002; Dannenmann et al. 2009) and of the diversity of the microbial community (Schulze et al. 2005; Koranda et al. 2011). Moreover, altered rhizodeposition by girdling can lead to a decreased microbial activity in the soil (Högberg and Read 2006). The sensitivity of soil microbial communities to the quantity and quality of C and N provided by tree roots is therefore highlighted by girdling experiments.

Aside from girdling experiments, the C flux from tree roots to soil micro-organisms can be altered by environmental factors. For example Ruehr et al. (2009) showed that in beech trees, C transfer to soil micro-organisms was significantly delayed under drought conditions. Moreover, it had been hypothesized that during a drought, adult beech trees could decrease

the activity of free-living soil microorganisms by reducing the quantity of rhizodeposits (Dannenmann et al. 2009). This decreased microbial activity could in return affect the nutritional status of the tree, either positively by shifting the competitive balance for N in favour of the tree rather than soil microorganisms (Dannenmann et al. 2009), or negatively by decreasing the microbial mineralisation activity (Kreuzwieser and Geßler 2010), resulting in altered nutrient contents in trees (Sardans et al. 2008a; Sardans et al. 2008b).

During drought-induced tree declines, a potential impairment of the C transfer to the roots and soil might therefore lead to changes in the soil microbial community of the rhizosphere, which in turn could affect the nutritional status of the trees. However, the relationships between tree decline and the C flux to roots and soil is still very poorly documented. The study of a case of unexplained *Eucalyptus* decline has shown that the dimished crown health of the trees was associated with a modification of the soil functional diversity, which could indicate an alteration of the chemical composition of rhizodeposits in declining trees (Cai et al. 2010).

In this study, we wondered if the decline of mature beech trees observed at the crown level could be related to alterations in the belowground compartment. We investigated two aspects of the belowground soil-plant continuum: root-related features (rooting depth, root density and root growth), and rhizodeposition-related features (quantity and quality of rhizodeposits). More precisely, we asked the following questions:

(i) Is tree decline associated with altered root features (density and growth)?

(ii) Can the quantity and/or quality of rhizodeposits be linked with tree decline?

As rhizodeposition cannot be assessed directly, we relied on several proxies to estimate the quantity and the quality of rhizodeposits. Root soluble organic carbon (Marchand 2003), rhizospheric soil soluble organic carbon (Haynes and Francis 1993), and microbial biomass (Haynes and Francis 1993) were used as proxies of the quantity of rhizodeposits. Bacterial

abundances in the rhizospheric soil were used both as a proxy for the quantity of rhizodeposits and the quality of rhizodeposits, by discriminating between copiotroph and oligotroph bacteria (Dennis et al. 2010). The functional diversity of soil bacteria was used as an indicator of the chemical diversity of rhizodeposits (Baudoin et al. 2002).

Material and methods

Site description and sampling design

The study was carried out in Fontainebleau state forest, France (48°22' N, 02°36' E, mean elevation 120 m a.s.l.) during the year 2013. This forest extends over 17, 000 ha, 60 km southeast of Paris. The climate is temperate, with a mean annual temperature of 10.6°C and mean annual precipitations of 749 mm, well distributed throughout the year (average on the 1960-2010 period). The study site is a mature, monospecific beech (*Fagus sylvatica* L.) stand, where mean tree age in 2011 was 95 years and dominant height 27 m, showing signs of decline since the late 1990's. Trees were growing on a rather shallow soil (mean depth 0.6 m), with a C/N ratio of 18.7 and extractible soil water content of 101 mm.

Five healthy and five declining trees were selected using the DEPEFEU (DEPErissement des FEUillus) protocol (Nageleisen and Goudet 2011), developed by the French National Forest Office (Office National des forêts, ONF). These two categories corresponded to trees with dense crowns and abundant fine ramification (healthy trees), and with very transparent crowns, only one or two main branches remaining and very limited fine ramification (declining trees). The soil profile was characterized at the foot of each tree. Two different sets of sampling were performed: one for root density and growth measurements, and one for the characterization of the biological activity in the rhizopheric soil.

Root density and growth

To assess root density and growth, three ingrowth cores per tree were set up ca. 30 cm away from the stem of each tree in January (2013/01/16). The three cores were evenly distributed around the stem. The cores were 10 cm in diameter, and 30 cm deep. They were divided in three 10 cm thick layers immediately after sampling. The segmented cores were placed in a cooler, and brought back to the laboratory for treatment. Within three days from the sampling date, roots were separated from the soil by hand and washed in tap water to remove as much soil and debris as possible. Roots were separated in three categories: coarse roots (more than 5 mm in diameter), medium roots (diameter between 2 mm and 5 mm) and fine roots (less than 2 mm in diameter) (Le Goff and Ottorini 2001).The soil was then sieved at 2 mm, and put back in place within a week from the sampling date. The roots were freeze-dried during three days, and weighed for dry weight. In July (2013/07/10) and December (2013/12/17), this procedure was repeated in the same ingrowth cores. The roots contained by the cores at these two dates therefore grew during the January-July and July-December intervals.

Soil sampling for soil microbial activity measurements

In March (2013/03/22), June (2013/06/18) and November (2013/11/04), two superficial rhizospheric soil samples (between 0 and 10cm deep) per tree were taken. Rhizospheric soil was defined as the soil that remained adherent to the roots when gently shaken. The two samples were taken at diametrically opposed location, ca.30 cm from the stem. The samples were brought back to the laboratory in a cooler, and processed during the following week. Roots and rhizospheric soil were separated, and the soil samples were sieved at 2mm. The sieves were thoroughly rinsed with tap water, and then cleaned with 90% ethanol between samples.

Microbial biomass and soluble organic C in soil and fine roots

For soil soluble organic C concentration measurements, rhizospheric soil samples were dried for 12 hours at 37°C and finely ground with a ball mill (MM301, Retsch, Germany). 5g of soil were extracted with 25 ml of distilled water for 18 hours at 80°C. For soluble organic C in roots, root samples were freeze dried for 3 days and ground to fine powder with a ball mill (MM301, Retsch, Germany). 0.5 g of root powder were extracted with 15 ml of distilled water at 60°C.

Microbial biomasses were estimated by the C and N content of the microbial community from fresh soil samples using the chloroform extraction-fumigation method (Vance et al. 1987). Briefly, for each soil sample, two 7.5g aliquots were weighted. One of the two aliquots was fumigated with chloroform at 25°C, in the dark during 24 h, while the other one was not subjected to the fumigation treatment. The organic C of both aliquots was extracted with a K_2SO_4 solution (0.5M).

The solutions resulting from the extractions described above were filtered on Whatman GF/C glass microfiber filters (GE Healthcare, UK). Dissolved organic C and N were analyzed in a Total Organic Carbon analyzer (Shimadzu ON-LINE TOC-V_{CSH} with optional TNM-1 , Tokyo, Japan). The results were expressed as g of organic C or N per g of dry soil.

Cultivable soil bacteria

4 g of fresh sieved rhizospheric soil were extracted with 40 ml of phosphate buffer (7.2 g NaCl, 2.8 g Na_2HPO_4, 0.4 g KH_2PO_4 l^{-1} water, pH 7·2) during 10 minutes at room temperature, and shaken at 40 rpm. The resulting solution was centrifuged at 750 rpm during 10 minutes. Phosphate buffer was then added to the resulting supernatant to obtain 10^{-2} to 10^{-5} successive dilutions. 100µl of the dilutions from 10^{-3} to 10^{-5} were spread onto agar plates (Tryptic Soy Agar, Sigma-Aldrich, France) to estimate the number of cultivable bacteria,

quantified as colony-forming units (CFU). Two replicates were prepared for each dilution. The agar plates were incubated in the dark at 27°C. CFU were counted two days (fast-growing bacteria), and ten days (slow-growing bacteria) after inoculation. The results were expressed as log CFU per g dry soil.

Soil functional bacterial diversity

To estimate the functional diversity of the microbial community in the rhizosphere, catabolic profiles were established using Biolog Ecoplates™ (Biolog, USA). 150µl of the 10-3 dilution used for the cultivable bacteria quantification were placed in each well of a Biolog Ecoplate™. The plates were incubated in the dark at 27°C, and the color development in the wells was followed every 24 hours with a microplate reader (Σ960, Metertech, Taiwan) at 550 nm. The results were expressed as Average Well Color Development (AWCD), richness (the number of wells with color development), Shannon's diversity index (H) and Shannon's evenness index (E), as described in Zak et al. (1994), Gomez et al. (2006) and Cai et al. (2010).

Statistical analysis

The effect of health status was tested using Student's t tests. The potential differences between sampling dates was tested with pairwise paired Student's t test. All the statistical analyses were performed with R software (http://www.r-project.org/).

Results

Soil profiles and soil water content

The soil profile at the foot of each tree is shown in Table IV.1. The soil profiles of healthy trees all corresponded to typical luvisols, ca. 1 m deep. The soil profiles of declining trees were more variable, ranging from shallow luvisols (trees 109D and 110D, Table IV.1) to deeper ocric podzosols showing a clear chocolate-coloured BPhs horizon above an eluvation (E) horizon (Table IV.1).

		Health status										
		Healthy					Declining					
		Trees										
Depth of the horizons (cm)	Horizon	1S	6S	9S	14S	16S	107D	109D	110D	112D	113D	pH
	OL	2	2	2	1	1	1	2	1	1	2	3
	OF	5	4	3	2	2	2	3	2	2	3	
	OH	NA	6	5	NA	NA	4	5	4	3	5	
	A	14	13	10	12	19	8	10	10	6	12	
	BPhs	NA	NA	NA	NA	NA	20	NA	NA	20	25	
	E	30	50	82	73	40	45	20	30	40	50	
	B_t	80	80	100	88	65	76	35	45	70	80	
	^{II}C	90	90	150	90	80	85	40	50	85	NA	7

Table IV.1 Depth of the base of the different soil horizons and soil pH at the foot of healthy and declining beech trees growing in the Fontainebleau state forest. The absence of some horizons is indicated by NAs.

Root growth

In January, when ingrowth cores were set up, healthy and declining trees had similar fine and coarse root densities, whatever the depth (Figure IV.1). Between 10 and 20 cm deep, declining trees had however significantly less medium roots than healthy trees (P<0.05, Figure IV.1b). In July and December, there were no medium and coarse roots: only fine roots had grown during the time intervals. Between January and July, declining trees produced more roots than healthy trees between 0 and 10 cm (P<0.05, Figure IV.1a) and between 20 and 30 cm (P<0.05, Figure IV.1c). Between July and December, healthy and declining trees

produced the same amount of fine roots, whatever the depth (Figure IV.1). When fine root mass at the different depths were added up, healthy and declining trees had similar fine root densities in January. Between January and July, declining trees produced more fine roots than healthy trees, but between July and December, fine root production was similar in both health statuses.

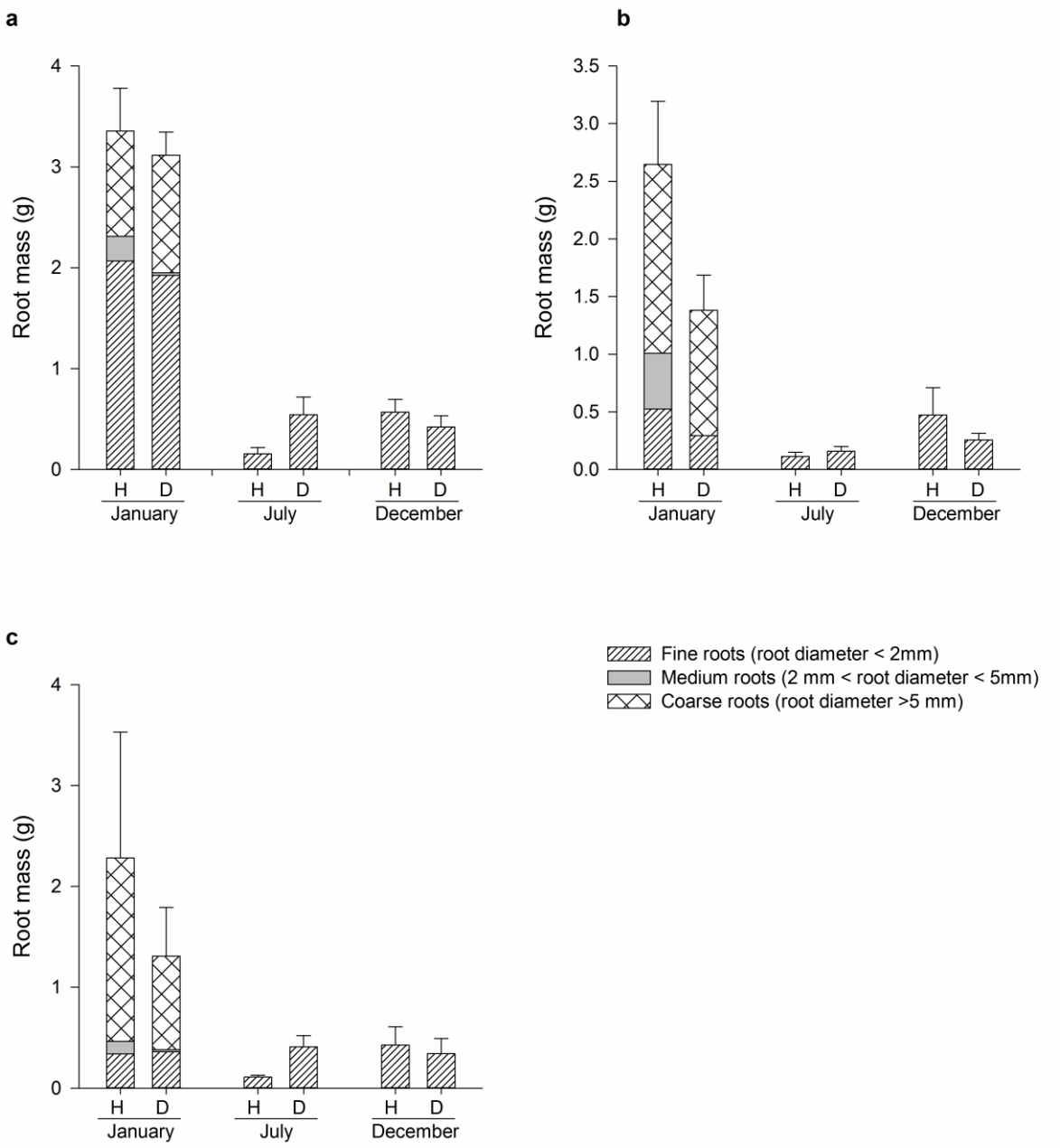

Figure IV.1 Average root mass (in g) of healthy and declining beech trees growing in the Fontainebleau state forest (France) in January (at the onset of ingrowth cores), July and December, between 0 and 10 cm (a), 10 and 20 cm (b), and 20 and 30 cm (c). The error bars represent standard errors.

Soluble organic carbon in soil and roots

The soluble organic carbon concentration in the soil was not affected by health status (Table IV.2). The concentrations in March were significantly higher than in June and November (P<0.05). The concentrations were similar in June and November (Table IV.2).

In roots, the soluble organic carbon concentrations were similar in healthy and declining trees (Table IV.2). The soluble organic carbon concentration was increased significantly between March and June (P<0.0001), and the concentrations in June and November were similar (Table IV.2).

	Date	Healthy trees	Declining trees
Soil soluble organic C (mg g dry soil^{-1})	March	3.14 ± 0.81	3.72 ± 0.53
	June	2.39 ± 0.12	2.66 ± 0.07
	Novembre	1.93 ± 0.29	2.00 ± 0.19
Root soluble organic C (mg g dry matter^{-1})	March	2.08 ± 0.37	1.59 ± 0.10
	June	6.70 ± 1.59	8.01 ± 1.05
	Novembre	7.87 ± 0.87	7.19 ± 1.28
Microbial biomass C (mg C g dry soil^{-1})	March	2.64 ± 0.52	2.12 ± 0.31
	June	1.51 ± 0.21	1.12 ± 0.19
	Novembre	-	-
Microbial biomass N (mg N g dry soil^{-1})	March	0.08 ± 0.01	0.07 ± 0.01
	June	0.11 ± 0.01	0.09 ± 0.01
	November	0.05 ± 0.01	0.06 ± 0.01
C:N ratio of the microbial biomass	March	33.13 ± 4.33	30.34 ± 1.99
	June	13.63 ± 0.83	12.55 ± 1.28
	November	-	-

Table IV.2 Average (± standard errors) soil water content, soil soluble organic carbon, root soluble organic carbon and microbial biomass in the rhizospheric soil of healthy and declining beech trees growing in the Fontainebleau state forest (France) in March, June and November.

Carbon in the microbial biomass

Similar amounts of C and N were contained in the microbial biomass of the rhizospheric soil of healthy and declining trees whatever the date (Table IV.2). The C content of the microbial biomass in November could not be measured due to technical difficulties. The C:N ratio of the microbial biomass was similar for healthy and declining trees in March and June

(Table IV.2). The C and N content and the C:N ratio of the microbial biomass varied significantly between sampling dates. The C content of the microbial biomass significantly increased between March and June (P<0.05). The N content of the microbial biomass significantly increased between March and June (P<0.05), and then decreased between June and November (P<0.001). The C:N content of the microbial biomass significantly decreased between March and June (P<0.05).

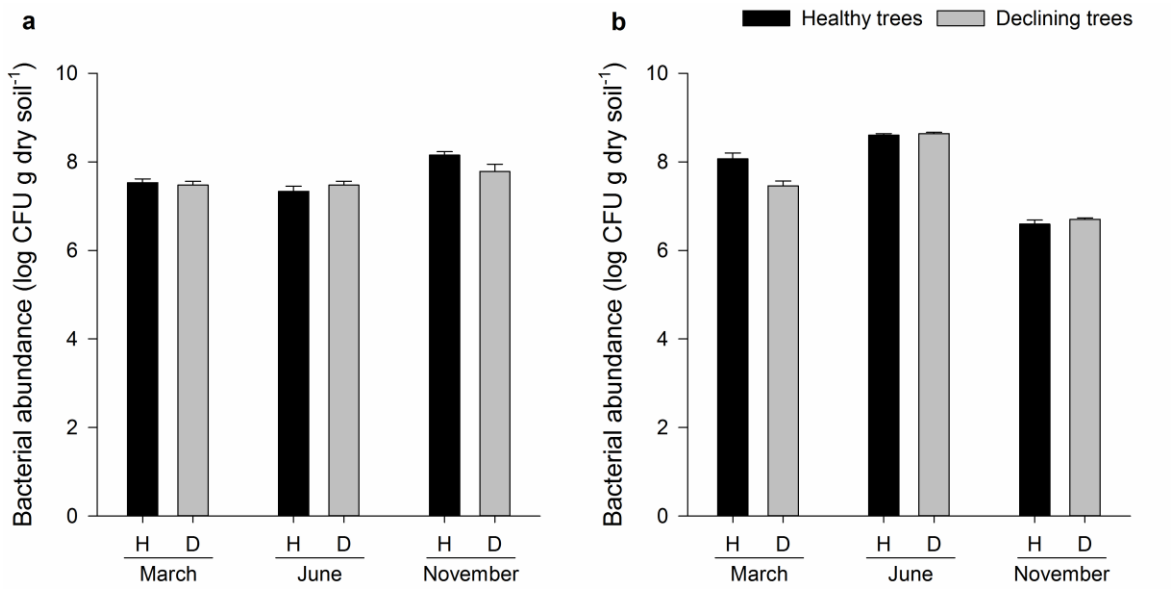

Figure IV.2 Average abundances (in log CFU g soil^{-1}) of fast-growing (a) and slow-growing (b) bacteria in the rhizospheric soil of healthy (black) and declining (grey) beech trees growing in the Fontainebleau state forest (France) in March, June and November. The error bars represent standard errors.

Cultivable bacteria counts

In March and June, healthy and declining trees had similar amounts of fast-growing cultivable bacteria in the rhizospheric soil (Figure IV.2a). However, in November, declining trees had significantly less fast-growing cultivable bacteria in the rhizospheric soil than healthy trees (P<0.05, Figure IV.2a). Healthy trees had similar amounts of fast-growing cultivable bacteria in the rhizospheric soil in March and June, and this amount increased in November. In declining trees, the amounts of fast-growing cultivable bacteria were the same at all dates (Figure IV.2a). Declining trees had less slow-growing bacteria in the rhizospheric

soil than healthy trees in March (P<0.01). However, both health statuses had similar amounts of slow-growing bacteria in June and November (Figure IV.2b). The amounts of slow-growing bacteria increased between March and June (P<0.0001), and decreased during the second part of the growing season (P<0.0001, Figure IV.2b).

Functional bacterial diversity

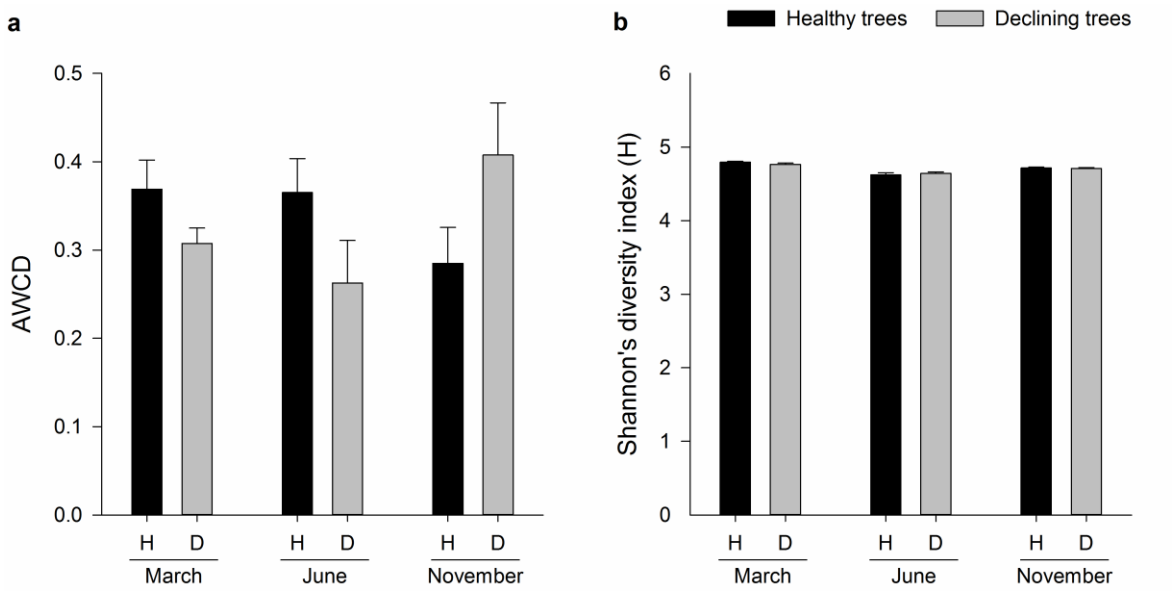

Figure IV.3 Measures of the functional bacterial diversity in the rhizospheric soil of healthy (black) and declining (grey) beech trees growing in the Fontainebleau state forest (France) in March, June and November: Average Well Color Development (AWCD, a) and Shannon's diversity index (H, b). The error bars represent standard errors.

Declining trees tended to have a lower AWCD in March and June, and a higher AWCD in November than healthy trees. This difference was however not statistically significant. Nevertheless, the AWCD of the rhizospheric bacterial communities of healthy and declining trees had contrasted temporal dynamics (Figure IV.3a). In healthy trees, AWCD decreased consistently between March and November, whereas in declining trees, it decreased between March and June, and increased between June and November (Figure IV.3a). Shannon's diversity index (H) was not affected by health status, whatever the date (Figure IV.3b). Shannon's diversity index showed small, but significant variation during the growing season:

it decreased between March and June, and increased between June and November ($P<0.0001$, Figure IV.3b). Healthy and declining trees used mainly the same carbon sources, but at different intensities (Table IV.2). Carbon sources used by only one of the two health statuses were usually some of the least used (Table IV.3).

	March		June		November	
Rank	Healthy trees	Declining trees	Healthy trees	Declining trees	Healthy trees	Declining trees
1	E	E	E	E	I	E
2	G	G	M	I	E	F
3	B	F	D	D	F	M
4	I	I	F	M	B	B
5	F	B	H	H	D	I
6	D	D	I	F	L	D
7	M	M	Q	L	G	G
8	C	N	A	G	M	H
9	H	H	L	B	H	Q
10	N	L	N	A	A	N
11	U	O	R	U	C	L
12	A	C	G	Q	N	C
13	L	U	C	N	K	R
14	J	J	B	R	U	A
15	O	A	U	C		K
16	S	P	K			U
17	Q		P			T
18	P					O
19						P
20						S

Table IV.3 Ranks of used carbon sources in healthy and declining beech trees growing in the Fontainebleau state forest (France). A carbon source is considered significantly used when its average color development is above 0.25. Green indicates carbon sources for which healthy and declining trees share the same rank. Yellow indicates a carbon sources that are used by both healthy and declining trees, but at different ranks. Red indicates that carbon sources used only by one of the two health statuses. Each letter represents a carbon source. A list of the carbon sources corresponding to each letter is available in the supplementary data (Table IV.A1).

Discussion

Soil types

The soil profiles under healthy and declining trees are typical of the evolution of soils formed on stampian sands enriched in fine particles in this area (Robin 1990). The coarse texture of this substrate favours nutrient leaching. Moreover, the beech litter promotes the podzolisation process on this substrate by producing massive amounts of soluble organic compounds that alter the structure of clay (Duchaufour 1977; Robin 1993). Due to the nature of clay minerals in Fontainebleau, this alteration releases Fe^{3+} and Al^{3+} ions. The strong spatial variability of soil profiles on this type of substrate has been shown to be related to the depth of a limestone layer beneath the stampian sand: soils show less signs of podzolisation where the limestone layer is closest to the soil surface (Robin 1990).

Root densities and growth

The majority of the fine root mass was found in the upper 10 cm of soil, which is coherent with previous observations (Mainiero and Kazda 2006). These superficial roots have been shown to be of particular importance in response to drought. Indeed, they enable rapid water transport when the soil is wet, but disconnect the tree from the driest soil patched when drought-induced embolism develops (Alder et al. 1996; Martínez-Vilalta et al. 2002).

Initial fine root densities were not significantly affected by tree decline (Figure IV.1). This finding is coherent with results obtained on beech, which showed that the size of the fine root system is only weakly influenced by soil fertility and acidity (Leuschner et al. 2004). Therefore, even if the soil profiles under healthy and declining trees are different (Table IV.1), these differences probably had no effect on fine root densities. Declining trees had however no medium roots between 10 and 20 cm below the surface in January (Figure IV.1b). This absence could be related to the presence of a BPhs horizon at these depths under some of

the declining trees (Table IV.1). Indeed, an accumulation of Al and Fe ions is typically observed in BPhs horizons (Duchaufour 1977). In large quantities, these ions are toxic for plant roots, negatively affecting root growth and function (Oleksyn et al. 1996; Kochian et al. 2005). Therefore, it can be hypothesized that the adverse soil properties between 10 and 20 cm deep under declining trees hindered the exploration of this layer of soil by tree roots.

During the first half of the year, declining trees produced more fine roots than healthy trees in the 0-10 and 20-30 cm layers (Table IV.1). An increased root production in the humic layer was also observed in *Picea abies* growing on acidic soils, with fine roots productions in acidic soils being twice those of less acidic soils (Jentschke et al. 2001). In *Picea abies* however, root growth decreased lower in the profile (Jentschke et al. 2001), which contradicts our observations. Stronger root growth has been associated in beech to more acidic topsoil pH (Leuschner and Hertel 2003). This enhanced root growth in acidic and nutrient poor sites can be interpreted as a response to low nutrient availability in soils with a low biological activity (Vogt et al. 1995). This relation cannot however explain the stronger fine root growth we observed in declining trees, as topsoil pH are similar in healthy and declining trees (Data not shown). Nevertheless, fine root production and turnover has been suggested to be a sensitive indicator of changing soil environments (Leuschner and Hertel 2003). The enhanced fine root growth of declining trees during the first part of the growing season could therefore reflect differences in soil properties (for example in terms of nutrient availability) under healthy and declining trees. These differences need to be further investigated in the near future.

Quantity and quality of rhizodeposits

Due to the complex nature of the soil environment, the quantity and quality of rhizodeposits cannot be assessed directly. Therefore, different proxies were used to study these variables. Root exudation was estimated by the root soluble organic content (Marchand

2003). Exudates are however not the only source of rhizodeposits: mucilage and sloughed-off cells and tissue from living roots, and soluble lysates and volatile compounds from damaged cells are also significant contributors of rhizodeposition (Hirsch et al. 2013). Moreover, the influence of root exudates on the microbial community of the rhizosphere is probably limited to a small spatiotemporal window (Hirsch et al. 2013). To estimate the importance of the C flux from tree roots to the soil, it is therefore necessary to take into account the other sources of rhizodeposits. As rhizodeposition is a major source of substrates for the microorganisms in the rhizosphere (Lynch and Whipps 1990), indicators of microbial abundances can be used as a proxy for the quantity of rhizodeposits. The microbial biomass as estimated with the extraction-fumigation technique is one such indicator (Vance et al. 1987). The microbial biomass in the rhizospheric soil of healthy and declining trees was similar (Table IV.2). This first indicator therefore suggests that the rhizodeposition of healthy and declining trees is quantitatively similar. This result is further confirmed by the similar soluble organic C concentrations in the rhizospheric soil of healthy and declining trees (Table IV.2). Indeed, the soluble organic C in the rhizospheric soil is mainly of microbial origin, and is considered as an indicator of the quantity of rhizodeposits (Haynes and Francis 1993). The abundance of cultivable bacteria can also be used as an indicator of the quantity, but also of the quality of rhizodeposits (Dennis et al. 2010). In March declining trees had less slow-growing bacteria than healthy ones, and the same observation was made in November for fast-growing bacteria (Figure IV.2). These differences could suggest slight differences in the quantity and/or composition of rhizodeposits during periods of low C transfer by the tree to the soil (Epron et al. 2011). The composition of rhizodeposits can further be investigated through catabolic profiles of the microbial community (Grayston and Campbell 1996; Baudoin et al. 2002). The overall catabolic activity (AWCD) of the bacterial community of the rhizospheric soil obtained with Biolog Ecoplates® tended to be lower in declining trees in March and June, and

higher in November compared to healthy trees. This resulted in a contrasted seasonal evolution in healthy and declining trees (Figure IV.3a). The decreased bacterial activity in the rhizospheric soil of declining trees could be related to different environmental conditions in the soil under trees of contrasted health status. Indeed, the thinner canopies of declining trees allow higher irradiance levels to reach the ground. This could lead to a greater variability of soil temperature and/or water availability in the vicinity of declining trees, particularly during the summer months, contributing to a decreased biological activity in the rhizosphere of declining trees. Contrarily to observations made in *Eucalyptus* (Cai et al. 2010), this different microbial activity was however not associated with different Shannon's diversity indexes (Figure IV.3b), and the bacterial communities in the rhizosphere of healthy and declining trees used mainly the same substrates (Table IV.3). The functional diversity of the rhizospheric bacterial communities was therefore not related to tree decline, and the composition of the rhizodeposits of healthy and declining trees is likely to be mostly similar.

Overall, the proxies of the quality and the quantity of rhizodeposits indicate no difference in the quality and quantity of rhizodeposits in healthy and declining trees. The seasonal variations of the quantity and quality of rhizodeposition are therefore stronger than the variations induced by the health status of the trees. This is in agreement with results from a girdling experiment, that showed that the phenological variations of rhizodeposition were stronger than those induced by limiting the C flux to roots and soil by girdling in beech (Rasche et al. 2011). Indeed, during a girdling experiment, the impaired phloem transport caused less variations of the phylogenetic composition of archeal and bacterial communities than the seasonal variation of rhizodeposition (Rasche et al. 2011).

In the case of decline studied, the C flux to roots and soil does not seem to be impaired in the reported case of tree decline. On the contrary, the C flux to roots seems to be slightly increased in declining trees during the first half of the year, as indicated by the fine root

growth data. However, root growth and rhizodeposition were estimated locally. Healthy and declining trees could thus display different spatial patterns of root growth and/or rhizodeposition undetected in this study.

Many environmental factors influence the rhizodeposition, both qualitatively and quantitatively. Among those factors, some nutrient deficiencies of the tree (particularly iron and phosphate) are known to increase rhizodeposition (Ryan et al. 2001; Hirsch et al. 2013). We hypothesized that the increased root growth of declining trees could indicate a lower nutrient availability in the rhizospheric soil of declining trees. As the rhizodeposition of healthy and declining trees is quantitatively similar, it can be hypothesized that the potentially limited nutrient availability does not result in stress-inducing deficiencies in declining trees compared to healthy ones. Nevertheless, this aspect of tree decline needs to be further investigated, as nutrient imbalances (for example N, Mg, Ca and K) have been associated in the past to tree decline cases (Schulze 1989; Aber et al. 1998). This is particularly relevant regarding the soil type difference between healthy and declining trees. Indeed, podzolic soils tend to have lower base cations contents than luvisols (Robin 2005). Mineral nutrition might however not be a decisive triggering factor, but rather a predisposing factor interacting with other environmental conditions such as droughts (Becker and Levy 1988).

References

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen Saturation in Temperate Forest Ecosystems. Bioscience 48:921–934.

Alder NN, Sperry JS, Pockman WT (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in Acer grandidentatum populations along a soil moisture gradient. Oecologia 105:293–301.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell NG, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A, Cobb NS (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684.

Anderegg WRL, Anderegg LDL, Berry JA, Field C (2014) Loss of whole-tree hydraulic conductance during severe drought and multi-year forest die-off. Oecologia 175:11–23.

Baudoin E, Benizri E, Guckert A (2002) Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl Soil Ecol 19:135–145.

Becker M, Levy G (1988) A propos du dépérissement des forêts : climat, sylviculture et vitalité de la sapinière vosgienne. Rev For Française 40:345–358.

Bréda N, Badeau V (2008) Forest tree responses to extreme drought and some biotic events: Towards a selection according to hazard tolerance? Comptes Rendus Geosci 340:651–662.

Cai YF, Barber PA, Dell B, O'Brien P, Williams N, Bowen B, Hardy G (2010) Soil bacterial functional diversity is associated with the decline of Eucalyptus gomphocephala. For Ecol Manage 260:1047–1057.

Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kögel-Knabner I, Knicker H, Mayer H, Schloter M, Pena R, Polle A, Rennenberg H, Papen H (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol Biochem 41:1622–1631.

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327.

Duchaufour P (1977) Pédologie 1. Pédogénèse et classification. Masson, Paris.

Ekberg A, Buchmann N, Gleixner G (2007) Rhizospheric influence on soil respiration and decomposition in a temperate Norway spruce stand. Soil Biol Biochem 39:2103–2110.

Epron D, Ngao J, Dannoura M, Bakker MR, Zeller B, Bazot S, Bosc A, Plain C, Lata JC, Priault P, Barthes L, Loustau D (2011) Seasonal variations of belowground carbon transfer assessed by in situ 13CO2 pulse labelling of trees. Biogeosciences 8:1153–1168.

Le Goff N, Ottorini J-M (2001) Root biomass and biomass increment in a beech (Fagus sylvatica L.) stand in North-East France. Ann For Sci 58:1–13.

Gomez E, Ferreras L, Toresani S (2006) Soil bacterial functional diversity as influenced by organic amendment application. Bioresour Technol 97:1484–1489.

Grayston SJ, Campbell CD (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (Larix eurolepis) and Sitka spruce (Picea sitchensis). Tree Physiol 16:1031–1038.

Grayston SJ, Vaughan D, Jones DL (1997) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56.

Haynes RJ, Francis GS (1993) Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. J Soil Sci 44:665–675.

Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310.

Hinsinger P, Bengough A, Vetterlein D, Young I (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152.

Hirsch PR, Miller AJ, Dennis PG (2013) Do Root Exudates Exert More Influence on Rhizosphere Bacterial Community Structure Than Other Rhizodeposits? In: Molecular Microbial Ecology of the Rhizosphere. John Wiley & Sons, Inc., pp 229–242.

Högberg MN, Chen Y, Högberg P (2007) Gross nitrogen mineralisation and fungi-to-bacteria ratios are negatively correlated in boreal forests. Biol Fertil Soils 44:363–366.

Högberg MN, Högberg P (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol 154:791–795.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792.

Högberg P, Read DJ (2006) Towards a more plant physiological perspective on soil ecology. Trends Ecol Evol 21:548–554.

Intergovernmental Panel on Climate Change (2014) Climate Change 2014: Synthesis Report.

Jentschke G, Drexhage M, Fritz H-W, Fritz E, Schella B, Lee D-H, Gruber F, Heimann J, Kuhr M, Schmidt J, Schmidt S, Zimmermann R, Godbold D (2001) Does soil acidity reduce subsoil rooting in Norway spruce (Picea abies)? Plant Soil 237:91–108.

Kochian L V., Piñeros MA, Hoekenga OA (2005) The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. In: Lambers H, Colmer T (eds) Root Physiology: from Gene to Function SE - 9. Springer Netherlands, pp 175–195.

Koranda M, Schnecker J, Kaiser C, Fuchslueger L, Kitzler B, Stange CF, Sessitsch A, Zechmeister-Boltenstern S, Richter A (2011) Microbial processes and community composition in the rhizosphere of European beech - The influence of plant C exudates. Soil Biol Biochem 43:551–558.

Kreuzwieser J, Gessler A (2010) Global climate change and tree nutrition: influence of water availability. Tree Physiol 30:1221–1234.

Leuschner C, Hertel D (2003) Fine Root Biomass of Temperate Forests in Relation to Soil Acidity and Fertility, Climate, Age and Species. In: Esser K, Lüttge U, Beyschlag W, Hellwig F (eds) Progress in Botany SE - 16. Springer Berlin Heidelberg, pp 405–438.

Leuschner C, Hertel D, Schmid I, Koch O, Muhs A, Hölscher D (2004) Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil 258:43–56.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manage 259:698–709.

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10.

Mainiero R, Kazda M (2006) Depth-related fine root dynamics of Fagus sylvatica during exceptional drought. For Ecol Manage 237:135–142.

Manion PD (1981) Tree disease concepts. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.

Marchand A-L (2003) Etude de la rhizodéposition chez le maïs : influence de la distribution des photoassimilats aux racines et de la morphologie racinaire. Institut National Polytechnique de Lorraine

Martínez-Vilalta J, Piñol J, Beven K (2002) A hydraulic model to predict drought-induced mortality in woody plants: an application to climate change in the Mediterranean. Ecol Modell 155:127–147.

McDowell NG (2011) Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. Plant Physiol 155:1051–1059. doi: 10.1104/pp.110.170704

McDowell NG, Pockman WT, Allen CD, Breshears DD, Cobb NS, Kolb T, Plaut JA, Sperry JS, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality

during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739.

Nageleisen L-M, Goudet M (2011) Manuel de Notation des dommages forestiers (symptômes, causes, état des cimes). Paris.

Oleksyn J, Karolewski P, Giertych MJ, Werner A, Tjoelker MG, Reich PB (1996) Altered root growth and plant chemistry ofPinus sylvestris seedlings subjected to aluminum in nutrient solution. Trees 10:135–144.

Rasche F, Knapp D, Kaiser C, Koranda M, Kitzler B, Zechmeister-Boltenstern S, Richter A, Sessitsch A (2011) Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. ISME J 5:389–402.

Robin A-M (1990) Les sols sur sables soufflés de la forêt de Fontainebleau…et leur fragilité. Bull la Société Bot Fr Lettres Bot 137:211–220.

Robin A-M (1993) Catalogue des principales stations forestières de la forêt de Fontainebleau. :371.

Robin A-M (2005) Épisodes majeurs de la podzolisation en forêt de Fontainebleau (France). Essai de synthèse à l'aide du radiocarbone naturel. Comptes Rendus Geosci 337:599–608.

Ruehr NK, Offermann CA, Geßler A, Winkler JB, Ferrio JP, Buchmann N, Barnard RL (2009) Drought effects on allocation of recent carbon: from beech leaves to soil CO2 efflux. New Phytol 184:950–961.

Ryan PPR, Delhaize E, Jones DDL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:527–560.

Sala A, Piper FI, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol 186:274–281.

Sardans J, Peñuelas J, Ogaya R (2008a) Drought-Induced Changes in C and N Stoichiometry in a Quercus ilex Mediterranean Forest. For Sci 54:513–522.

Sardans J, Peñuelas J, Ogaya R (2008b) Drought's impact on Ca, Fe, Mg, Mo and S concentration and accumulation patterns in the plants and soil of a Mediterranean evergreen Quercus ilex forest. Biogeochemistry 87:49–69.

Schulze E-D (1989) Air Pollution and Forest Decline in a Spruce (Picea abies) Forest. Sci VO - 244:776.

Schulze W, Gleixner G, Kaiser K, Guggenberger G, Mann M, Schulze E-D (2005) A proteomic fingerprint of dissolved organic carbon and of soil particles. Oecologia 142:335–343.

Sevanto S, Mcdowell NG, Dickman LT, Pangle R, Pockman WT (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell Environ 37:153–161.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707.

Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H (1995) Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant Soil 187:159–219.

Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154:327–338.

Zak JC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: A quantitative approach. Soil Biol Biochem 26:1101–1108.

Supplementary data

Carbon source	Corresponding letter
β-methyl-D-glucoside	A
D-galactonic acid γ lactone	B
L-arginine	C
Pyruvic acid Methyl ester	D
D-galacturonic acid	E
L-asparagine	F
Tween 40	G
Tween 80	H
D-mannitol	I
2-hydroxy benzoic acid	J
4-hydroxy benzoic acid	K
L-serine	L
N-acetyl-D-glucosamide	M
D-glucosaminic acid	N
Itaconic acid	O
Glycyl-L-glutamic acid	P
D-cellobiose	Q
Glucose-1-phosphate	R
α-D-lactose	S
D,L-α-glycerol phosphate	T
Putrescine	U
I-erythritol	
L-phenylalanine	
α-cyclodextrine	
γ-hydroxybutyric acid	
L-threonine	
Glycogen	
α-ketobutyric acid	
Phenylethyl-amine	
D-malic acid	
D-xylose	

Table IV.A1 List of the individual carbon sources of an Ecoplate®, with the corresponding letters used in table IV.3. No letter was attributed to the substrates that were not significantly used in the study.

V. Discussion générale et perspectives

Au cours de ce travail, nous avons documenté les mécanismes fonctionnels sous-jacents à un dépérissement à long terme de hêtres adultes en forêt de Fontainebleau. Ce dépérissement est lié à des sécheresses répétées, probablement en association avec des conditions édaphiques défavorables (Chapitre IV). Le dépérissement a ici été étudié en dehors de fortes contraintes. Ce sont donc des arrières-effets de stress passés qui ont été étudiés.

Dans la littérature, un manque de substrats carbonés disponibles pour le métabolisme et/ou un dysfonctionnement du système hydraulique sont les deux principaux mécanismes fonctionnels avancés pour expliquer les dépérissements et la mortalité liés à des stress hydriques (McDowell et al. 2008). Si une abondante littérature s'est récemment développée sur le sujet, elle s'est néanmoins essentiellement concentrée sur les conséquences à court et moyen termes de sécheresses sur le fonctionnement des arbres. Les arrières effets à long terme de sécheresses répétées sont par conséquent mal connus. De plus, le rôle potentiel d'autres aspects importants du fonctionnement des arbres lors d'un dépérissement restent jusqu'ici inexplorés, comme par exemple celui des réserves azotés et du flux de carbone vers les racines et le sol. Afin de mieux comprendre les mécanismes fonctionnels sous-jacents aux dépérissements à long terme, nous avons choisi d'adopter une approche intégrant les aspects hydraulique, carboné et azoté du fonctionnement du système arbre-sol. L'étude s'est déroulée sur trois années (2011, 2012 et 2013), dont une (2011) a présenté un stress hydrique modéré au printemps. Les principaux résultats obtenus sont résumés dans la figure V.1.

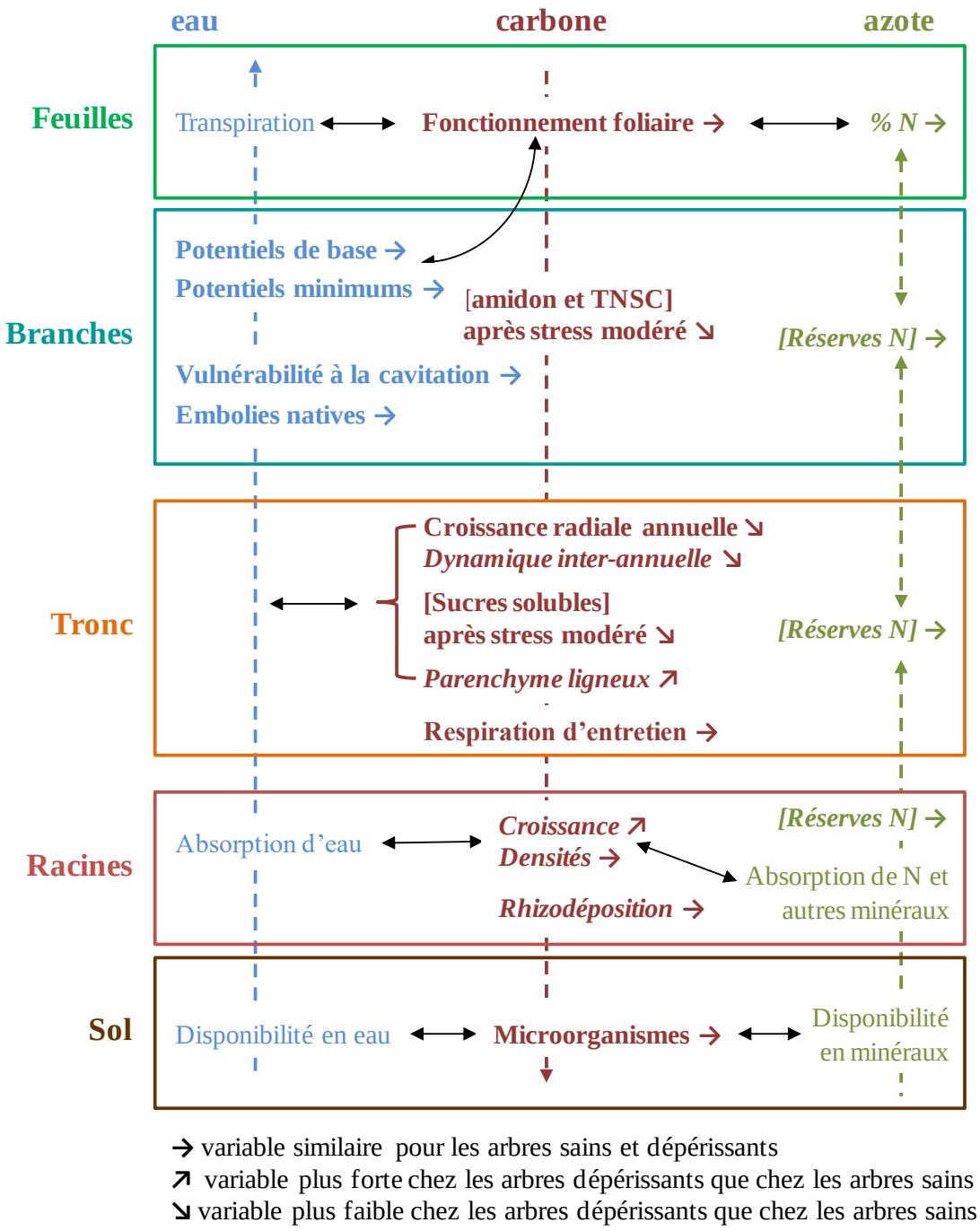

Figure V.1 Représentation schématique des aspects du fonctionnement du système arbre-sol documentés au cours de ce travail. Les variables étudiées sont en gras, et les aspects novateurs par rapport à la littérature existante sont en gras et italique. Les flèches en pointillé indiquent les transferts dans le système arbre-sol, et les flèches noires indiquent les principaux points de couplage discutés dans ce travail.

En dehors de fortes contraintes, l'état hydrique et le fonctionnement hydraulique des arbres sains et dépérissants sont similaires (Chapitre II). De même, les concentrations et les dynamiques des réserves azotées ainsi que la respiration d'entretien ne sont pas affectées par

le statut sanitaire de l'arbre (Chapitres II et III). En revanche, la croissance radiale des arbres dépérissants est plus faible, présente une variation interannuelle diminuée et une dynamique intra-annuelle modifiée par rapport à celles des arbres sains (Chapitre II). Parallèlement à cette croissance radiale diminuée, on observe une croissance racinaire plus rapide chez les arbres dépérissants durant la première moitié de l'année, associée à des sols globalement plus podzolisés au pied des arbres dépérissants. La quantité et la qualité des rhizodépots ne semblent par contre pas varier en fonction du statut sanitaire de l'arbre (Chapitre IV). Les teneurs en réserves carbonées des arbres dépérissants sont diminuées (particulièrement dans les branches fines) par rapport à celles des arbres sains après une contrainte hydrique modérée, mais montrent leur capacité de résilience en remontant à des niveaux similaires à ceux des arbres sains après une année favorable (Chapitres II et III). Dans le xylème, on observe chez les arbres dépérissants une augmentation de la proportion de tissus de stockage (parenchyme ligneux), associée à une modification de la structure des rayons ligneux (Chapitre III).

En conditions non contraignantes, le fonctionnement des arbres dépérissants à l'échelle de l'organe semble peu modifié par rapport à celui des sains, à l'exception de la nette diminution de la croissance radiale du tronc. Une croissance radiale réduite avait déjà été rapportée sur les arbres dépérissants de la parcelle étudiée (Silva 2010). Cette observation est cohérente avec la littérature : lors d'événements de dépérissement ou de mortalité, une diminution de la croissance radiale est en effet une caractéristique souvent associée à l'apparition à plus ou moins long terme de symptômes de dépérissements et/ou à une augmentation de la probabilité de mourir de l'arbre (Torelli et al. 1986; Jenkins and Pallardy 1995; Pedersen 1998; Demchik and Sharpe 2000; Amoroso et al. 2012). Quelques exceptions sont cependant rapportées dans la littérature. Ainsi, sur un site de sapins pectinés

(*Abies alba* Mill.) dépérissants, il a été observé que les arbres dépérissants avaient des taux de croissance plus forts que les arbres sains (Cailleret et al. 2013).

Plusieurs facteurs pourraient expliquer une diminution de croissance radiale.

Premièrement, l'état hydrique des arbres dépérissants pourrait être à l'origine d'une limitation directe de la croissance radiale. En effet, une limitation de la disponibilité en eau peu induire une diminution de la pression de turgescence (Hsiao 1973), ce qui affecte négativement le processus d'expansion cellulaire (Hsiao 1973; Zweifel et al. 2006). Dans notre cas, il semble peu probable que des facteurs hydriques expliquent la croissance radiale plus faible des arbres dépérissants par rapport à celle des arbres sains. En effet, les mesures de potentiels de base et de potentiels minimum montrent qu'en dehors de périodes de stress, la contrainte hydrique subie par les arbres sains et dépérissants semble similaire.

Une carence en azote peut également être avancée pour expliquer la diminution de croissance des arbres dépérissants. L'azote peut en effet imposer une limitation directe à la croissance des arbres (Morot-Gaudry 1997). Bien que nous n'ayons pas spécifiquement documenté l'état de nutrition azotée de nos arbres, les concentrations et dynamiques similaires des réserves azotées, associées aux proportions semblables en azote des feuilles de lumière ne semblent pas indiquer de carence azotée. Néanmoins, des résultats complémentaires obtenus au printemps 2015 font apparaitre un contenu en azote plus faible dans la biomasse microbienne du sol rhizosphérique des arbres dépérissants. Ce résultat, en contraste avec ceux obtenus au cours de l'année 2013, suggère une plus faible disponibilité de l'azote dans sol rhizosphérique des arbres dépérissants. En effet, le contenu en azote de la biomasse microbienne est lié au contenu en azote des substrats disponibles pour les micro-organismes (Wardle 1998). De plus, des sols podzolisés, dans lesquels l'azote est un élément particulièrement limitant (Tamm and Pettersson 1969), ont été mis en évidence au pied des arbres dépérissants. Une plus faible disponibilité de l'azote dans le sol rhizosphérique des

arbres dépérissants serait par ailleurs cohérente avec les croissances racinaires plus fortes des arbres dépérissants durant la première moitié de l'année.

La croissance réduite des arbres dépérissants pourrait être également liée à une modification de l'allocation du carbone à l'échelle de l'individu. En effet, des expériences de défoliation ont montré que lors de stress, l'allocation du carbone au réserves pourrait se trouver priorisée par rapport à la croissance (Wiley et al. 2013; Saffell et al. 2014). Cette priorisation des réserves par rapport à la croissance pourrait favoriser la survie des arbres à des stress plus sévères et/ou plus prolongés (Wiley et al. 2013). Récemment, une telle priorisation de la formation de réserves carbonées par rapport à la croissance a également été mise en évidence en conditions de sécheresse sévère à mise en place progressive chez des jeunes sapins (Hartmann et al. 2015). Une des caractéristiques marquantes des arbres dépérissants étudiés par rapport aux arbres sains est la plus faible réponse de leur croissance radiale aux variations interannuelles de climat, à laquelle s'ajoute la résilience de leurs réserves carbonées au cours de l'année 2012 (Figure V.2). **Chez les arbres dépérissants, le remplissage saisonnier des réserves (particulièrement dans les organes de remobilisation à faible distance comme les branches fines) répond donc de manière plus forte aux variations interannuelles de climat que la croissance radiale du tronc**. L'inverse est observé chez les arbres sains. On peut donc se demander si, chez les arbres dépérissants, la formation de réserves pourrait se trouver priorisée par rapport à la croissance radiale. Cette priorisation pourrait se faire soit par une régulation directe de la formation de réserves, soit via une régulation de la croissance radiale (Dietze et al. 2014).

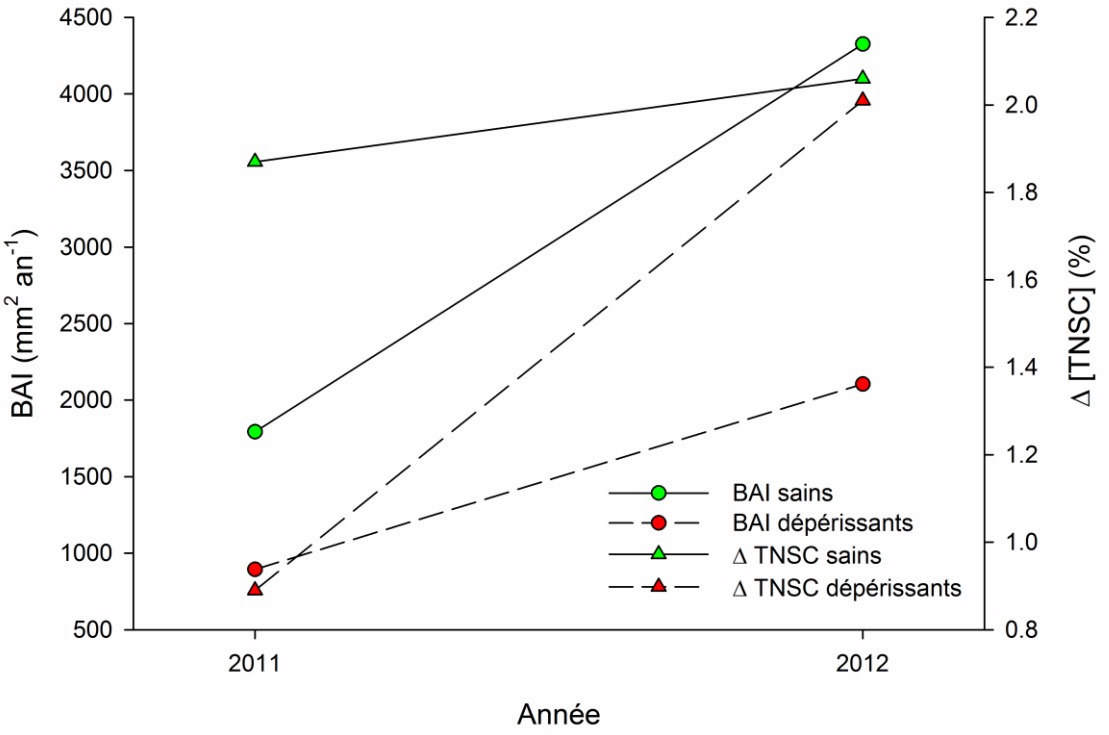

Figure V.2 Variations de l'incrément annuel en surface du tronc (Basal Area Increment, BAI) et du remplissage saisonnier des réserves carbonées dans le xylème du tronc (Δ TNSC) en 2011 et 2012 chez des hêtres sains et dépérissants matures en forêt de Fontainebleau.

La résilience des réserves carbonées dans les branches fines des arbres dépérissants pourrait par ailleurs avoir des implications en matière de fonctionnement hydraulique. En effet, le maintien de concentrations suffisantes en réserves carbonées pourrait être important pour assurer la régulation osmotique de la sève brute (Sevanto et al. 2014). La récupération observée des réserves carbonées dans les branches des arbres dépérissants est rendue possible grâce à l'alternance entre années contraignantes et années favorables. On peut alors se demander si, en cas de sécheresses plus sévères ou répétées, les concentrations en réserves carbonées des arbres dépérissants pourraient passer sous un seuil problématique, par exemple en-dessous duquel la régulation osmotique de la sève brute ne peut plus être assurée. Malgré leurs caractéristiques hydrauliques similaires à celles des arbres sains (Chapitre II), les branches des arbres dépérissants pourraient alors présenter des taux d'embolie problématiques

pour le fonctionnement de l'arbre plus rapidement que les branches des arbres sains en cas de sécheresse sévère ou répétée. Par ailleurs, Lemoine et al. (2002) ont montré chez le hêtre une forte variabilité de l'architecture hydraulique au sein des houppiers, liée à la position des branches dans le houppier et à leur exposition lumineuse : les branches les plus haut situées et les plus exposées à la lumière présentent ainsi une conductivité hydraulique spécifique et une résistance à la cavitation plus élevées (Lemoine et al. 2002). La forte mortalité de branches fines que nous avons observée dans la partie supérieure du houppier des arbres dépérissants modifie l'exposition à la lumière des branches restantes, et conduit à une descente de cime progressive. Il serait donc intéressant de quantifier la variabilité des caractéristiques hydrauliques des branches au sein des houppiers des arbres sains et dépérissants.

De manière plus générale, le dépérissement pourrait impacter la proportion du carbone assimilé alloué aux grandes fonctions de l'arbre. En effet, les arbres dépérissants présentent une surface foliaire totale très réduite, et des caractéristiques foliaires semblables à celles des arbres sains. Par conséquent, l'assimilation totale de carbone à l'échelle de l'arbre se trouve probablement diminuée chez les arbres dépérissants, comme cela a déjà été montré chez des peupliers (Anderegg et al. 2014). Or, il apparait dans nos résultats que certains puits de carbone ne semblent pas diminués par le dépérissement. Ainsi, la respiration d'entretien par unité de surface de tronc n'est pas affectée par le dépérissement, et les troncs des arbres sains et dépérissants sont de taille comparable. Il est donc probable que la respiration d'entretien à l'échelle des troncs entiers soit quantitativement similaire chez les arbres sains et dépérissants. De même, la rhizodéposition ne semble pas affectées par le dépérissement, et la croissance des racines fines des arbres dépérissants est augmentée par rapport à celle des sains. **On peut donc se demander si, à l'échelle de l'arbre, la proportion du carbone assimilé allouée à**

toutes ces fonctions ne pourrait pas être plus grande chez les arbres dépérissants, réduisant alors la proportion de carbone alloué à la croissance radiale du tronc.

Dans cette perspective, **la croissance radiale pourrait être considérée comme la principale variable d'ajustement à long terme des arbres dépérissants à une assimilation réduite de carbone**. La réduction des coûts carbonés liés à la croissance radiale du tronc passerait alors à la fois par une diminution de la quantité de bois formé (Figure V.2), mais également par une diminution de la respiration de croissance, comme le montrent des mesures effectuées en juin 2011 (Figure V.3). Cette plus faible respiration de croissance est due à la plus faible vitesse de croissance des arbres dépérissants (Ceschia et al. 2002) : en 2011, la respiration de croissance et la vitesse de croissance (maximale au moment des mesures présentées en Figure V.3) sont toutes les deux divisées par un facteur de 1.5.

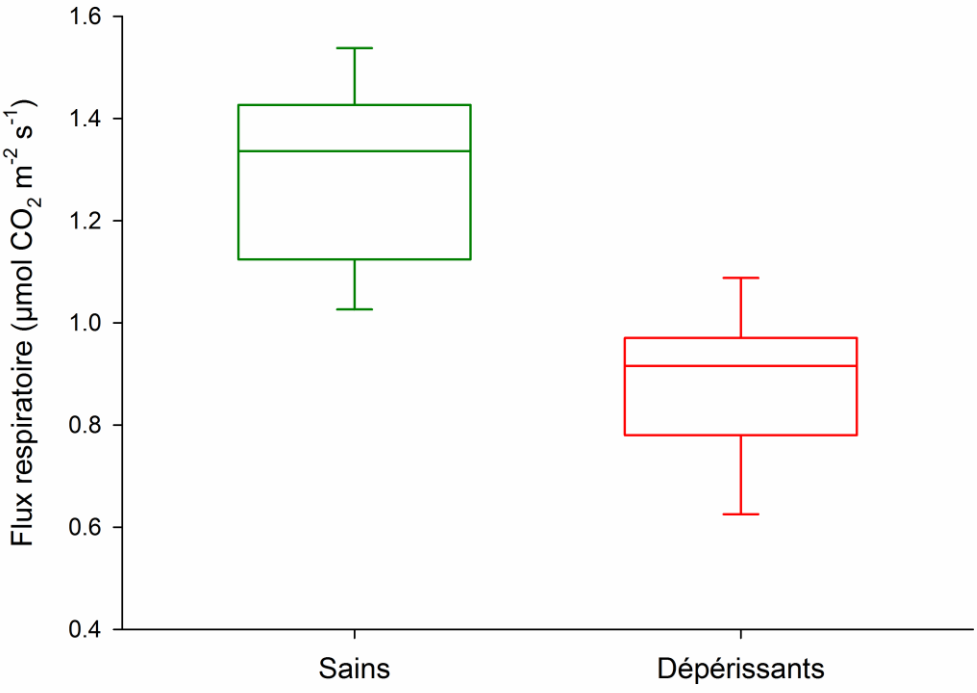

Figure V.3 Respiration de croissance des troncs de hêtres sains et dépérissants poussant en forêt de Fontainebleau en juin 2011, à 16°C. Les barres d'erreurs représentent les 5^{e} et 95^{e} percentiles.

Sur la base d'estimations de l'incrément en biomasse et en réserves carbonées à l'échelle de l'arbre entier, des travaux précédents sur un dépérissement de hêtres dans le nord-est de la France avaient également suggéré que dans un dépérissement de hêtres matures, la croissance était la variable d'ajustement à une assimilation réduite de carbone (Genet 2009). Pour confirmer cette hypothèse chez nos arbres, il sera nécessaire de calculer un bilan de carbone à l'échelle de l'arbre entier pour les arbres sains et dépérissants. Par ailleurs, il faut ici signaler que la reproduction et la synthèse de composés de défense, deux puits de carbone potentiellement importants dans la plante n'ont pas été évalués, ce qui limite d'autant plus l'extrapolation de nos résultats à l'échelle de l'arbre. Une approche de modélisation basée sur les processus, comme par exemple avec le modèle CASTANEA développé au sein de l'équipe Ecophysiologie Végétale du laboratoire « Ecologie, Systématique et Evolution », permettrait de tester certaines de ces hypothèses de modification de l'allocation du carbone à l'échelle de l'arbre et leurs implications à long terme sur la survie de l'arbre.

L'étude des largeurs de cernes menée par Silva (2010) sur la parcelle étudiée au cours de ce travail montre que la diminution de la croissance radiale des arbres actuellement dépérissants n'est pas un phénomène récent, puisqu'elle commence après la sécheresse de l'année 1959. Cette diminution de croissance radiale pourrait impacter le fonctionnement des arbres dépérissants, notamment du point de vue hydraulique. En effet, bien qu'à l'échelle des branches les caractéristiques hydrauliques des arbres sains et dépérissants soient similaires (Chapitre II), une telle diminution de la croissance radiale du tronc à long terme pourrait modifier la capacité des arbres dépérissants à assurer un transport d'eau suffisant, en réduisant la surface conductrice dans le tronc. Face à une capacité de conduction de l'eau dans le tronc diminuée, les arbres dépérissants ajusteraient alors leur surface transpirante (Bréda et al. 2006), conduisant à la détérioration observée de l'état du houppier. Pour explorer cette

hypothèse, il serait intéressant de connaitre l'évolution des flux de sève à différentes profondeurs dans le tronc et différentes hauteurs dans le houppier chez les arbres sains et dépérissants.

Par ailleurs, on peut se demander si la réduction de croissance observée s'accompagne d'une modification des caractéristiques fonctionnelles du bois. Dans le chapitre III, une augmentation de la proportion de parenchyme ligneux, associées à une modification de la forme des rayons ligneux, a été mise en évidence. L'impact fonctionnel de cette anatomie du bois modifiée reste à explorer. En effet, les rayons ligneux sont dans le xylème le lieu de stockage des réserves (Tromp 1983), et pourraient également avoir un rôle dans le maintien de l'intégrité du système hydraulique (Spicer 2014). Il reste à déterminer à quel moment dans l'histoire du dépérissement la différence de proportion de parenchyme et de forme des rayons ligneux s'est mise en place : précède-t-elle la diminution de croissance, ou bien est-elle ultérieure ? Plus largement, l'étude de caractéristiques anatomiques fonctionnelles dans les cernes, et en particulier de traits reliés au fonctionnement hydraulique (diamètre et épaisseur des parois des vaisseaux de xylème par exemple) en plus des caractéristiques liées au parenchyme ligneux nous permettrait d'affiner notre connaissance de l'histoire de la mise en place des symptômes de dépérissement de manière rétrospective.

De plus, des mesures effectuées au niveau du sol suggèrent un lien potentiel entre une plus faible disponibilité en nutriments dans le sol et le dépérissement (Chapitre IV). Les communautés microbiennes ont une influence sur la disponibilité des minéraux dans le sol (Van Der Heijden et al. 2008). Dans ce travail, la diversité fonctionnelle globale des bactéries de la rhizosphère a été documentée. Il serait intéressant dans le futur d'étudier plus précisément certains éléments de cette diversité fonctionnelle, notamment en ciblant la

diversité des bactéries spécifiques du cycle de l'azote. En outre, une étude actuellement en cours sur la structure des communautés microbiennes devrait nous permettre d'avoir une vision plus globale de la composition de ces communautés en prenant en compte les bactéries, les champignons et les archées.

La question de la nutrition minérale chez les arbres sains et dépérissants mériterait d'être approfondie dans le futur, notamment par des dosages d'éléments minéraux ayant dans le passé été liés à des dépérissements. Ainsi, des carences en calcium, magnésium, potassium, fer ou manganèse ont été documentées lors de la vague de dépérissement des années 1970-1980 (Schulze 1989). De plus, une toxicité liée à la présence de trop fortes concentrations en ions Al^{3+} a également été suspectée dans des cas précédents de dépérissement dans les années 1970-1980 (Godbold et al. 1988; Schulze 1989), mais également dans des cas de dépérissements plus récents (Genet 2009). L'implication actuelle de la nutrition minérale dans le dépérissement étudié ici pourrait être abordée en mesurant les concentrations des éléments minéraux d'intérêt dans le sol et différents organes des arbres sains et dépérissants (racines et feuilles en particulier). Comme pour les caractéristiques anatomiques fonctionnelles, il serait particulièrement intéressant de développer une approche rétrospective de la nutrition minérale des arbres sains et dépérissants en dosant les minéraux d'intérêt dans les cernes. Si cette approche est valable pour l'aluminium (Penninckx et al. 2001), des études précédentes ont en revanche montré que la composition en éléments minéraux des cernes de hêtres n'était pas un bon indicateur de la composition chimique de la solution du sol pour le calcium, le potassium et le magnésium en raison de processus internes de remobilisation (Herbauts et al. 2002). On peut néanmoins de demander si les cycles internes de ces minéraux sont modifiés par le dépérissement, par exemple via une altération des transferts radiaux dans les rayons ligneux.

En conclusion, dans le cadre du dépérissement à long terme de hêtres étudié, les principales différences fonctionnelles mises en évidence sont des modifications des dynamiques saisonnières et interannuelles de la croissance radiale du tronc et des réserves carbonées dans les branches. Pour mieux comprendre les implications fonctionnelles de ces différences, il serait important de transposer les résultats obtenus au cours de ce travail à l'échelle de l'arbre entier. Pour cela, une étape de biométrie sera indispensable, aucune relation allométrique valable n'étant disponible pour les arbres dépérissants. Par ailleurs, ce travail pourra également être prolongé par une approche dendrochronologique basée des traits fonctionnels dans les cernes des arbres sains et dépérissants, permettant d'affiner notre compréhension de la mise en place du dépérissement sur le long terme. Une autre piste intéressante à développer est l'implication potentielle de divergences fonctionnelles liées à l'interface arbre-sol, par exemple en termes de nutrition minérale ou de structure des communautés microbiennes rhizosphériques.

VI. Bibliographie

Aber J, McDowell W, Nadelhoffer K, et al. (1998) Nitrogen Saturation in Temperate Forest Ecosystems. Bioscience 48:921–934. doi: 10.2307/1313296

Adams HD, Germino MJ, Breshears DD, et al. (2013) Nonstructural leaf carbohydrate dynamics of Pinus edulis during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. New Phytol 197:1142–1151. doi: 10.1111/nph.12102

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, et al. (2009a) Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. Proc Natl Acad Sci U S A 106:7063–7066. doi: 10.1073/pnas.0901438106

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, et al. (2009b) Reply to Sala: Temperature sensitivity in drought-induced tree mortality hastens the need to further resolve a physiological model of death. Proc Natl Acad Sci U S A 106:E69. doi: 10.1073/pnas.0905282106

Alder NN, Sperry JS, Pockman WT (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in Acer grandidentatum populations along a soil moisture gradient. Oecologia 105:293–301. doi: 10.1007/BF00328731

Allen CD, Macalady AK, Chenchouni H, et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684. doi: 10.1016/j.foreco.2009.09.001

Amoroso MM, Daniels LD, Larson BC (2012) Temporal patterns of radial growth in declining Austrocedrus chilensis forests in Northern Patagonia: The use of tree-rings as an indicator of forest decline. For Ecol Manage 265:62–70.

Anderegg WRL (2012) Complex aspen forest carbon and root dynamics during drought. Clim Change 111:983–991 LA – English. doi: 10.1007/s10584-012-0421-9

Anderegg WRL, Anderegg LDL, Berry JA, Field C (2014) Loss of whole-tree hydraulic conductance during severe drought and multi-year forest die-off. Oecologia 175:11–23. doi: 10.1007/s00442-013-2875-5

Anderegg WRL, Berry JA, Smith DD, et al. (2012) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. Proc Natl Acad Sci U S A 109:233–237.

Badeau V (2007) Changements climatiques : ses conséquences pour la forêt française. Journées EUROFOREST. pp 75–90

Bahrman N, Plomion C, Petit RJ, Kremer A (1997) Contribution of two-dimensional electrophoresis of proteins to maritime pine genetics. Ann For Sci 54:225–236.

Barbaroux C, Bréda N (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. Tree Physiol 22:1201–1210.

Barbaroux C, Bréda N, Dufrêne É (2003) Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (Quercus petraea and Fagus sylvatica). New Phytol 157:605–615. doi: 10.1046/j.1469-8137.2003.00681.x

Barigah TS, Charrier O, Douris M, et al. (2013) Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. Ann Bot 112:1431–1437. doi: 10.1093/aob/mct204

Bastien Y (2000) Sylviculture du hêtre.

Baudoin E, Benizri E, Guckert A (2002) Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl Soil Ecol 19:135–145. doi: http://dx.doi.org/10.1016/S0929-1393(01)00185-8

Bazot S, Barthes L, Blanot D, Fresneau C (2013) Distribution of non-structural nitrogen and carbohydrate compounds in mature oak trees in a temperate forest at four key phenological stages. Trees - Struct Funct 27:1023–1034. doi: 10.1007/s00468-013-0853-5

Becker M, Levy G (1988) A propos du dépérissement des forêts : climat, sylviculture et vitalité de la sapinière vosgienne. Rev For Française 40:345–358.

Borghetti M, Leonardi S, Raschi A, et al. (1993) Ecotypic Variation of Xylem Embolism, Phenological Traits, Growth Parameters and Allozyme Characteristics in Fagus sylvatica. Funct Ecol 7:713–720.

Bossel H (1986) Dynamics of forest dieback: Systems analysis and simulation. Ecol Modell 34:259–288. doi: http://dx.doi.org/10.1016/0304-3800(86)90008-6

Bouriaud O, Bréda N, Le Moguédec G, Nepveu G (2004) Modelling variability of wood density in beech as affected by ring age, radial growth and climate. Trees - Struct Funct 18:264–276. doi: 10.1007/s00468-003-0303-x

Bower AD, Aitken SN (2006) Geographic and seasonal variation in cold hardiness of whitebark pine. Can J For Res 36:1842–1850. doi: 10.1139/x06-067

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Bréda N, Badeau V (2008) Forest tree responses to extreme drought and some biotic events: Towards a selection according to hazard tolerance? Comptes Rendus Geosci 340:651–662. doi: 10.1016/j.crte.2008.08.003

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought : a review of ecophysiological responses , adaptation processes and long-term consequences. Ann For Sci 63:625–644. doi: 10.1051/forest

Breshears DD, Myers OB, Meyer CW, et al. (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. Front Ecol Environ 7:185–189. doi: 10.1890/080016

Brodribb TJ, Bowman DJMS, Nichols S, et al. (2010) Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. New Phytol 188:533–542. doi: 10.1111/j.1469-8137.2010.03393.x

Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiol 149:575–584. doi: 10.1104/pp.108.129783

Brodribb TJ, Hill RS (1999) The importance of xylem constraints in the distribution of conifer species. New Phytol 143:365–372. doi: 10.1046/j.1469-8137.1999.00446.x

Brummer Y, Cui SW (2005) Understanding Carbohydrate Analysis. Food Carbohydrates Chem Phys Prop Appl. Taylor and Francis group, pp 67–104

Burgert I, Eckstein D (2001) The tensile strength of isolated wood rays of beech (Fagus sylvatica L.) and its significance for the biomechanics of living trees. Trees - Struct Funct 15:168–170. doi: 10.1007/s004680000086

Bussotti F, Prancrazi M, Matteucci G, Gerosa G (2005) Leaf morphology and chemistry in Fagus sylvatica (beech) trees as affected by site factors and ozone: results from CONECOFOR permanent monitoring plots in Italy. Tree Physiol 25:211–219. doi: 10.1093/treephys/25.2.211

Cai YF, Barber PA, Dell B, et al. (2010) Soil bacterial functional diversity is associated with the decline of Eucalyptus gomphocephala. For Ecol Manage 260:1047–1057.

Cailleret M, Nourtier M, Amm A, et al. (2013) Drought-induced decline and mortality of silver fir differ among three sites in Southern France. Ann For Sci 1–15. doi: 10.1007/s13595-013-0265-0

Ceschia É, Damesin C, Lebaube S, et al. (2002) Spatial and seasonal variations in stem respiration of beech trees (Fagus sylvatica). Ann For Sci 59:801–812. doi: 10.1051/forest

Chapin SF, Schultze E-D, Mooney HA (1990) The Ecology and Economics of Storage in Plants. Annu Rev Ecol Syst 21:423–447.

Charrier G, Améglio T (2011) The timing of leaf fall affects cold acclimation by interactions with air temperature through water and carbohydrate contents. Environ Exp Bot 72:351–357. doi: http://dx.doi.org/10.1016/j.envexpbot.2010.12.019

Charrier G, Bonhomme M, Lacointe A, Améglio T (2011) Are budburst dates, dormancy and cold acclimation in walnut trees (Juglans regia L.) under mainly genotypic or environmental control? Int J Biometeorol 55:763–774. doi: 10.1007/s00484-011-0470-1

Charrier G, Cochard H, Améglio T (2013) Evaluation of the impact of frost resistances on potential altitudinal limit of trees. Tree Physiol 33:891–902. doi: 10.1093/treephys/tpt062

Cheaib A, Badeau V, Boe J, et al. (2012) Climate change impacts on tree ranges: model intercomparison facilitates understanding and quantification of uncertainty. Ecol Lett 15:533–544.

Choat B, Jansen S, Brodribb TJ, et al. (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755.

Cochard H (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. Plant, Cell Environ 25:815–819. doi: 10.1046/j.1365-3040.2002.00863.x

Cochard H, Barigah ST, Kleinhentz M, Eshel A (2008) Is xylem cavitation resistance a relevant criterion for screening drought resistance among Prunus species? J Plant Physiol 165:976–982. doi: http://dx.doi.org/10.1016/j.jplph.2007.07.020

Cochard H, Herbette S, Barigah TS, et al. (2010) Does sample length influence the shape of xylem embolism vulnerability curves? A test with the Cavitron spinning technique. Plant Cell Environ 33:1543–1552. doi: 10.1111/j.1365-3040.2010.02163.x

Cochard H, Lemoine D, Améglio T, Granier A (2001) Mechanisms of xylem recovery from winter embolism in Fagus sylvatica. Tree Physiol 21:27–33.

Cruiziat P, Cochard H, Améglio T (2002) Hydraulic architecture of trees : main concepts and results. Ann For Sci 59:723–752. doi: 10.1051/forest

Damesin C, Ceschia É, Le Goff N, et al. (2002) Stem and branch respiration of beech: from tree measurements to estimations at the stand level. New Phytol 153:159–172.

Dannenmann M, Simon J, Gasche R, et al. (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol Biochem 41:1622–1631. doi: http://dx.doi.org/10.1016/j.soilbio.2009.04.024

Demchik MC, Sharpe WE (2000) The effect of soil nutrition, soil acidity and drought on northern red oak (Quercus rubra L.) growth and nutrition on Pennsylvania sites with high and low red oak mortality. For Ecol Manage 136:199–207. doi: http://dx.doi.org/10.1016/S0378-1127(99)00307-2

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327.

Déqué M (2007) Frequency of precipitation and temperature extremes over France in an anthropogenic scenario: Model results and statistical correction according to observed values. Glob Planet Change 57:16–26. doi: http://dx.doi.org/10.1016/j.gloplacha.2006.11.030

Dickman LT, McDowell NG, Sevanto S, et al. (2015) Carbohydrate dynamics and mortality in a piñon-juniper woodland under three future precipitation scenarios. Plant Cell Environ 38:729–739. doi: 10.1111/pce.12441

Dickson RE (1989) Carbon and nitrogen allocation in trees. Ann For Sci 46:631s–647s.

Dietze MC, Sala A, Carbone MS, et al. (2014) Nonstructural Carbon in Woody Plants. Annu Rev Plant Biol 65:667–687. doi: 10.1146/annurev-arplant-050213-040054

Dixon HH, Joly J (1984) On the Ascent of Sap. Proc R Soc London

Domec J-C, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees - Struct Funct 15:204–214. doi: 10.1007/s004680100095

Duan H, Amthor JS, Duursma RA, et al. (2013) Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO2] and elevated temperature. Tree Physiol 33:779–792. doi: 10.1093/treephys/tpt061

Duchaufour P (1977) Pédologie 1. Pédogénèse et classification. Masson, Paris

Dufrêne E, Davi H, François C, et al. (2005) Modelling carbon and water cycles in a beech forest: Part I: Model description and uncertainty analysis on modelled NEE. Ecol Modell 185:407–436. doi: http://dx.doi.org/10.1016/j.ecolmodel.2005.01.004

Durand-Gillmann M, Cailleret M, Boivin T, et al. (2012) Individual vulnerability factors of Silver fir (Abies alba Mill.) to parasitism by two contrasting biotic agents: mistletoe (Viscum album L. ssp. abietis) and bark beetles (Coleoptera: Curculionidae: Scolytinae) during a decline process. Ann For Sci 69:1–15. doi: 10.1007/s13595-012-0251-y

Ekberg A, Buchmann N, Gleixner G (2007) Rhizospheric influence on soil respiration and decomposition in a temperate Norway spruce stand. Soil Biol Biochem 39:2103–2110. doi: http://dx.doi.org/10.1016/j.soilbio.2007.03.024

Epron D, Ngao J, Dannoura M, et al. (2011) Seasonal variations of belowground carbon transfer assessed by in situ 13CO2 pulse labelling of trees. Biogeosciences 8:1153–1168. doi: 10.5194/bg-8-1153-2011

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19. doi: 10.1007/BF00377192

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537.

Fonti P, Tabakova M, Kirdyanov A, et al. (2015) Variability of ray anatomy of Larix gmelinii along a forest productivity gradient in Siberia. Trees - Struct Funct 1–11. doi: 10.1007/s00468-015-1197-0

Fotelli MN, Nahm M, Radoglou K, et al. (2009) Seasonal and interannual ecophysiological responses of beech (Fagus sylvatica) at its south-eastern distribution limit in Europe. For Ecol Manage 257:1157–1164.

Fotelli MN, Rennenberg H, Geßler A (2002) Effects of Drought on the Competitive Interference of an Early Successional Species (Rubus fruticosus) on Fagus sylvatica L.

Seedlings: 15N Uptake and Partitioning, Responses of Amino Acids and other N Compounds. Plant Biol 4:311–320. doi: 10.1055/s-2002-32334

Fotelli MN, Rienks M, Rennenberg H, Geßler A (2004) Climate and forest management affect 15N-uptake, N balance and biomass of European beech seedlings. Trees - Struct Funct 18:157–166. doi: 10.1007/s00468-003-0289-4

Galiano L, Martínez-Vilalta J, Lloret F (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. New Phytol 190:750–759. doi: 10.1111/j.1469-8137.2010.03628.x

Galiano L, Martínez-Vilalta J, Sabaté S, Lloret F (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiol 32:478–489. doi: 10.1093/treephys/tps025

Galván JD, Camarero JJ, Gutiérrez E (2014) Seeing the trees for the forest: drivers of individual growth responses to climate in Pinus uncinata mountain forests. J Ecol 102:1244–1257. doi: 10.1111/1365-2745.12268

Galvez D a, Landhäusser SM, Tyree MT (2011) Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation? Tree Physiol 31:250–257. doi: 10.1093/treephys/tpr012

Galvez DA, Landhäusser SM, Tyree MT (2013) Low root reserve accumulation during drought may lead to winter mortality in poplar seedlings. New Phytol 198:139–148. doi: 10.1111/nph.12129

Gansert D, Backes K, Kakubari Y (1999) Altitudinal and seasonal variation of frost resistance of Fagus crenata and Betula ermanii along the Pacific slope of Mt. Fuji, Japan. J Ecol 87:382–390. doi: 10.1046/j.1365-2745.1999.00354.x

Gartner BL, Baker DC, Spicer R (2000) Distribution and vitality of xylem rays in relation to tree leaf area in douglas fir. IAWA J 21:389–401. doi: 10.1163/22941932-90000255

Genet H (2009) Effets du vieillissement et de la fertilité minérale sur l'allocation du carbone entre la croissance, le stockage de réserves et la reproduction chez le chêne et le hêtre. Université Henri Poincaré Nancy 1

Gérard B, Bréda N (2012) Radial distribution of carbohydrate reserves in the trunk of declining European beech trees (Fagus sylvatica L.). Ann For Sci 69:1–8. doi: 10.1007/s13595-012-0240-1

Geßler A, Keitel C, Nahm M, Rennenberg H (2004) Water Shortage Affects the Water and Nitrogen Balance in Central European Beech Forests. Plant Biol 6:289–298.

Geßler A, Schneider S, Von Sengbusch D, et al. (1998) Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce (Picea abies) and beech (Fagus sylvatica) trees. New Phytol 138:275–285.

Gilson A, Barthes L, Delpierre N, et al. (2014) Seasonal changes in carbon and nitrogen compound concentrations in a Quercus petraea chronosequence. Tree Physiol 34:716–729. doi: 10.1093/treephys/tpu060

Godbold DL, Fritz E, Hüttermann A (1988) Aluminum toxicity and forest decline. Proc Natl Acad Sci U S A 85:3888–3892.

Le Goff N, Ottorini J-M (2001) Root biomass and biomass increment in a beech (Fagus sylvatica L.) stand in North-East France. Ann For Sci 58:1–13.

Gomez E, Ferreras L, Toresani S (2006) Soil bacterial functional diversity as influenced by organic amendment application. Bioresour Technol 97:1484–1489.

Gomez L, Faurobert M (2002) Contribution of vegetative storage proteins to seasonal nitrogen variations in the young shoots of peach trees (Prunus persica L. Batsch). J Exp Bot 53:2431–2439. doi: 10.1093/jxb/erf098

Grayston SJ, Campbell CD (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (Larix eurolepis) and Sitka spruce (Picea sitchensis). Tree Physiol 16:1031–1038. doi: 10.1093/treephys/16.11-12.1031

Grayston SJ, Vaughan D, Jones DL (1997) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56. doi: http://dx.doi.org/10.1016/S0929-1393(96)00126-6

Gruber A, Pirkebner D, Florian C, Oberhuber W (2011) No evidence for depletion of carbohydrate pools in Scots pine (Pinus sylvestris L.) under drought stress. Plant Biol (Stuttg) 14:142–148. doi: 10.1111/j.1438-8677.2011.00467.x

Hacke UG, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in Fagus sylvatica f. purpurea and Populus balsamifera. J Exp Bot 46:1177–1183. doi: 10.1093/jxb/46.9.1177

Hartmann H, McDowell NG, Trumbore S (2015) Allocation to carbon storage pools in Norway spruce saplings under drought and low CO2. Tree Physiol 35:243–252. doi: 10.1093/treephys/tpv019

Hartmann H, Ziegler W, Kolle O, Trumbore S (2013a) Thirst beats hunger - declining hydration during drought prevents carbon starvation in Norway spruce saplings. New Phytol 200:340–349. doi: 10.1111/nph.12331

Hartmann H, Ziegler W, Trumbore S (2013b) Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy. Funct Ecol 27:413–427. doi: 10.1111/1365-2435.12046

Haynes RJ, Francis GS (1993) Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. J Soil Sci 44:665–675. doi: 10.1111/j.1365-2389.1993.tb02331.x

Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. doi: 10.1111/j.1461-0248.2007.01139.x

Herbauts J, Penninckx V, Gruber W, Meerts P (2002) Radial variations in cation exchange capacity and base saturation rate in the wood of pedunculate oak and European beech. Can J For Res 32:1829–1837. doi: 10.1139/x02-097

Hinsinger P, Bengough A, Vetterlein D, Young I (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152. doi: 10.1007/s11104-008-9885-9

Hirsch PR, Miller AJ, Dennis PG (2013) Do Root Exudates Exert More Influence on Rhizosphere Bacterial Community Structure Than Other Rhizodeposits? Mol Microb Ecol Rhizosph. John Wiley & Sons, Inc., pp 229–242

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081.

Högberg MN, Chen Y, Högberg P (2007) Gross nitrogen mineralisation and fungi-to-bacteria ratios are negatively correlated in boreal forests. Biol Fertil Soils 44:363–366. doi: 10.1007/s00374-007-0215-9

Högberg MN, Högberg P (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol 154:791–795. doi: 10.1046/j.1469-8137.2002.00417.x

Högberg P, Nordgren A, Buchmann N, et al. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792.

Högberg P, Read DJ (2006) Towards a more plant physiological perspective on soil ecology. Trends Ecol Evol 21:548–554. doi: http://dx.doi.org/10.1016/j.tree.2006.06.004

Hsiao TC (1973) Plant Responses to Water Stress. Annu Rev Plant Physiol 24:519–570. doi: 10.1146/annurev.pp.24.060173.002511

Intergovernmental Panel on Climate Change (2014) Climate Change 2014: Synthesis Report.

Inventaire Forestier National (IFN) (2010) La forêt française. Les résultats issus des campagnes d'inventaire 2005 à 2009.

Jacquet J-S, Bosc A, O'Grady AP, Jactel H (2014) Combined effects of defoliation and water stress on pine growth and non-structural carbohydrates. Tree Physiol 34:367–376. doi: 10.1093/treephys/tpu018

Janská A, Maršík P, Zelenková S, Ovesná J (2010) Cold stress and acclimation – what is important for metabolic adjustment? Plant Biol 12:395–405. doi: 10.1111/j.1438-8677.2009.00299.x

Jenkins MA, Pallardy SG (1995) The influence of drought on red oak group species growth and mortality in the Missouri Ozarks. Can J For Res 25:1119–1127. doi: 10.1139/x95-124

Jentschke G, Drexhage M, Fritz H-W, et al. (2001) Does soil acidity reduce subsoil rooting in Norway spruce (Picea abies)? Plant Soil 237:91–108. doi: 10.1023/A:1013305712465

Johnson AH, Siccama TG (1983) Acid deposition and forest decline. Environ Sci Technol 17:294A–305A.

Kavanagh KL, Bond BJ, Aitken SN, et al. (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. Tree Physiol 19:31–37. doi: 10.1093/treephys/19.1.31

Klein T, Hoch G, Yakir D, Körner C (2014) Drought stress, growth and nonstructural carbohydrate dynamics of pine trees in a semi-arid forest. Tree Physiol 34:981–992. doi: 10.1093/treephys/tpu071

Kochian L V., Piñeros MA, Hoekenga OA (2005) The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. In: Lambers H, Colmer T (eds) Root Physiol from Gene to Funct SE - 9. Springer Netherlands, pp 175–195

Koranda M, Schnecker J, Kaiser C, et al. (2011) Microbial processes and community composition in the rhizosphere of European beech - The influence of plant C exudates. Soil Biol Biochem 43:551–558. doi: 10.1016/j.soilbio.2010.11.022

Krause GHM, Arndf U, Brandt CJ, et al. (1986) Forest decline in Europe : development and possible causes. Water, Air Soil Pollut 31:647–668.

Kreuzwieser J, Geßler A (2010) Global climate change and tree nutrition: influence of water availability. Tree Physiol 30:1221–1234. doi: 10.1093/treephys/tpq055

Kreyling J (2010) Winter climate change: a critical factor for temperate vegetation performance. Ecology 91:1939–1948. doi: 10.1890/09-1160.1

Landmann G (1994) Concepts, définitions et caractéristiques générales des dépérissements forestiers. Rev For Française 46:405–415.

Lebaube S, Goff N Le, Ottorini J-M, Granier A (2000) Carbon balance and tree growth in a Fagus sylvatica stand. Ann For Sci 57:49–61.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379. doi: 10.1890/06-2057.1

Lemoine D, Cochard H, Granier A (2002) Within crown variation in hydraulic architecture in beech (Fagus sylvatica L): evidence for a stomatal control of xylem embolism. Ann For Sci 59:19–27. doi: 10.1051/forest

Leuschner C, Hertel D (2003) Fine Root Biomass of Temperate Forests in Relation to Soil Acidity and Fertility, Climate, Age and Species. In: Esser K, Lüttge U, Beyschlag W, Hellwig F (eds) Prog Bot SE - 16. Springer Berlin Heidelberg, pp 405–438

Leuschner C, Hertel D, Schmid I, et al. (2004) Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil 258:43–56. doi: 10.1023/B:PLSO.0000016508.20173.80

Leuzinger S, Bigler C, Wolf A, Körner C (2009) Poor methodology for predicting large-scale tree die-off. Proc Natl Acad Sci U S A 106:E106. doi: 10.1073/pnas.0908053106

Lévy G, Becker M (1987) Le dépérissement du sapin dans les Vosges : rôle primordial de déficits d'alimentation en eau. Ann des Sci For 44:403–416.

Limousin J-M, Bickford CP, Dickman LT, et al. (2013) Regulation and acclimation of leaf gas exchange in a piñon-juniper woodland exposed to three different precipitation regimes. Plant Cell Environ 36:1812–1825. doi: 10.1111/pce.12089

Lindner M, Maroschek M, Netherer S, et al. (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manage 259:698–709. doi: http://dx.doi.org/10.1016/j.foreco.2009.09.023

Lindquist EJ, D'Annunzio R, Gerrand A, et al. (2012) Global forest land-use change 1990–2005. Rome

López BC, Gracia CA, Sabaté S, Keenan T (2009) Assessing the resilience of Mediterranean holm oaks to disturbances using selective thinning. Acta Oecologica 35:849–854. doi: http://dx.doi.org/10.1016/j.actao.2009.09.001

Lorenz M, Becher G (2012) Forest Condition in Europe 2012, Technical Report of ICP Forests. Hamburg

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10. doi: 10.1007/BF00011685

Maherali H, Pockman WT, Jackson RB (2004) Adaptative variation in the vulnerability of woody plants to xylem cavitation. Ecology 85:2184–2199. doi: 10.1890/02-0538

Mainiero R, Kazda M (2006) Depth-related fine root dynamics of Fagus sylvatica during exceptional drought. For Ecol Manage 237:135–142. doi: 10.1016/j.foreco.2006.09.034

Manion PD (1981) Tree disease concepts. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA

Marchand A-L (2003) Etude de la rhizodéposition chez le maïs : influence de la distribution des photoassimilats aux racines et de la morphologie racinaire. Institut National Polytechnique de Lorraine

Martínez-Vilalta J, Piñol J, Beven K (2002) A hydraulic model to predict drought-induced mortality in woody plants: an application to climate change in the Mediterranean. Ecol Modell 155:127–147. doi: http://dx.doi.org/10.1016/S0304-3800(02)00025-X

Maunoury-Danger F, Fresneau C, Eglin T, et al. (2010) Impact of carbohydrate supply on stem growth, wood and respired CO2 delta13C: assessment by experimental girdling. Tree Physiol 30:818–830. doi: 10.1093/treephys/tpq039

McDowell NG (2011) Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. Plant Physiol 155:1051–1059. doi: 10.1104/pp.110.170704

McDowell NG, Beerling DJ, Breshears DD, et al. (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. Trends Ecol Evol 26:523–532. doi: 10.1016/j.tree.2011.06.003

McDowell NG, Pockman WT, Allen CD, et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739. doi: 10.1111/j.1469-8137.2008.02436.x

McDowell NG, Sevanto S (2010) The mechanisms of carbon starvation: how, when, or does it even occur at all ? New Phytol 186:263–264.

Michelot A (2011) Croissance et delta 13C des cernes de trois essences forstières tempérées (Fagus sylvatica, Quercus petraea et Pinus sylvestris) face aux variations climatiques à l'échelle interannuelle et saisonière. Université Paris-Sud

Millard P, Grelet G-A (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. Tree Physiol 30:1083–1095. doi: 10.1093/treephys/tpq042

Mitchell PJM, O'Grady AP, Tissue DT, et al. (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytol 197:862–872. doi: 10.1111/nph.12064

Montwé D, Spiecker H, Hamann A (2014) An experimentally controlled extreme drought in a Norway spruce forest reveals fast hydraulic response and subsequent recovery of growth rates. Trees - Struct Funct 28:891–900. doi: 10.1007/s00468-014-1002-5

Mormiche A (1994) La gestion du dépérissement du hetre en normandie 1961-1988. Rev For Française 47:586–590.

Morot-Gaudry J-F (1997) Assimilation de l'azote chez les plantes - Aspects physiologique, biochimique et moléculaire, INRA. Paris

Moulinier J, Lorenzetti F, Bergeron Y (2014) Growth and mortality of trembling aspen (Populus tremuloides) in response to artificial defoliation. Acta Oecologica 55:104–112. doi: http://dx.doi.org/10.1016/j.actao.2013.12.007

Mueller-Dombois D (1988) Forest decline and dieback - A global ecological problem. Trends Ecol Evol 3:310–312. doi: 10.1016/0169-5347(88)90108-5

Nageleisen L, Reuter J (2007) Bilan de la santé des forêts en 2006.

Nageleisen L-M (1994) Le dépérissement actuel de feuillus divers : hêtre, merisier, alisier torminal, érable sycomore, peuplier, châtaignier, charme, aulne glutineux. Rev For Française 46:554–561.

Nageleisen L-M, Goudet M (2011) Manuel de Notation des dommages forestiers (symptômes, causes, état des cimes). Paris

Nardini A, Battistuzzo M, Savi T (2013) Shoot desiccation and hydraulic failure in temperate woody angiosperms during an extreme summer drought. New Phytol 200:322–329. doi: 10.1111/nph.12288

Ögren E (2000) Maintenance respiration correlates with sugar but not nitrogen concentration in dormant plants. Physiol Plant 108:295–299.

Ögren E (2001) Effects of climatic warming on cold hardiness of some northern woody plants assessed from simulation experiments. Physiol Plant 112:71–77. doi: 10.1034/j.1399-3054.2001.1120110.x

Oleksyn J, Karolewski P, Giertych MJ, et al. (1996) Altered root growth and plant chemistry ofPinus sylvestris seedlings subjected to aluminum in nutrient solution. Trees 10:135–144. doi: 10.1007/BF02340765

Oliet JA, Puértolas J, Planelles R, Jacobs DF (2013) Nutrient loading of forest tree seedlings to promote stress resistance and field performance: A Mediterranean perspective. New For 44:649–669. doi: 10.1007/s11056-013-9382-8

Påhlsson AM (1992) Influence of nitrogen fertilization on minerals, carbohydrates, amino acids and phenolic compounds in beech (Fagus sylvatica L.) leaves. Tree Physiol 10:93–100.

Pedersen BS (1998) The role of stress in the mortality of midwestern oaks as indicated by growth prior to death. Ecology 79:79–93.

Penninckx V, Glineur S, Gruber W, et al. (2001) Radial variations in wood mineral element concentrations: a comparison of beech and pedunculate oak from the Belgian Ardennes. Ann For Sci 58:253–260.

Penning de Vries FWT (1975) The Cost of Maintenance Processes in Plant Cells. Ann Bot 39:77–92.

Peuke AD, Rennenberg H (2004) Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (Fagus sylvatica L.): phosphorus most affected by drought. Trees - Struct Funct 18:639–648. doi: 10.1007/s00468-004-0335-x

Pilegaard K, Hummelshøj P, Jensen NO, Chen Z (2001) Two years of continuous CO2 eddy-flux measurements over a Danish beech forest. Agric For Meteorol 107:29–41. doi: http://dx.doi.org/10.1016/S0168-1923(00)00227-6

Piper FI (2011) Drought induces opposite changes in the concentration of non-structural carbohydrates of two evergreen Nothofagus species of differential drought resistance. Ann For Sci 68:415–424. doi: 10.1007/s13595-011-0030-1

Plaut JA, Yepez EA, Hill J, et al. (2012) Hydraulic limits preceding mortality in a piñon–juniper woodland under experimental drought. Plant Cell Environ 35:1601–1617.

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran Desert vegetation. Am J Bot 87:1287–1299.

Polge H (1966) Établissement des courbes de variation de la densité du bois par exploration densitométrique de radiographies d'échantillons prélevés à la tarière sur des arbres vivants : applications dans les domaines Technologique et Physiologique. Ann des Sci For 23:1–206. doi: 10.1051/forest/19660101

Poorter H, Niinemets Ü, Poorter L, et al. (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 182:565–588. doi: 10.1111/j.1469-8137.2009.02830.x

Poot P, Veneklaas EJ (2012) Species distribution and crown decline are associated with contrasting water relations in four common sympatric eucalypt species in southwestern Australia. Plant Soil 364:409–423. doi: 10.1007/s11104-012-1452-8

Pratt RB, Ewers FW, Lawson MC, et al. (2005) Mechanisms for tolerating freeze–thaw stress of two evergreen chaparral species: Rhus ovata and Malosma laurina (Anacardiaceae). Am J Bot 92:1102–1113. doi: 10.3732/ajb.92.7.1102

Pratt RB, Jacobsen AL, Mohla R, et al. (2008) Linkage between water stress tolerance and life history type in seedlings of nine chaparral species (Rhamnaceae). J Ecol 96:1252–1265. doi: 10.1111/j.1365-2745.2008.01428.x

Rasche F, Knapp D, Kaiser C, et al. (2011) Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. ISME J 5:389–402.

Ritchie GA, Hinckley TM (1975) The Pressure Chamber as an Instrument for Ecological Research. Adv Ecol Res Volume 9:165–254. doi: http://dx.doi.org/10.1016/S0065-2504(08)60290-1

Robin A-M (1990) Les sols sur sables soufflés de la forêt de Fontainebleau…et leur fragilité. Bull la Société Bot Fr Lettres Bot 137:211–220. doi: 10.1080/01811797.1990.10824880

Robin A-M (1993) Catalogue des principales stations forestières de la forêt de Fontainebleau. 371.

Robin A-M (2005) Épisodes majeurs de la podzolisation en forêt de Fontainebleau (France). Essai de synthèse à l'aide du radiocarbone naturel. Comptes Rendus Geosci 337:599–608. doi: 10.1016/j.crte.2005.01.008

Rodriguez-Calcerrada J, Lopez R, Salomon R, et al. (2015) Stem CO2 efflux in six co-occurring tree species: underlying factors and ecological implications. Plant Cell Environ 38:1104–1115. doi: 10.1111/pce.12463

Rosas T, Galiano L, Ogaya R, et al. (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Front Plant Sci 4:1–16. doi: 10.3389/fpls.2013.00400

Ruehr NK, Offermann CA, Geßler A, et al. (2009) Drought effects on allocation of recent carbon: from beech leaves to soil CO2 efflux. New Phytol 184:950–961. doi: 10.1111/j.1469-8137.2009.03044.x

Ryan MG (1990) Growth and maintenance respiration in stems of Pinuscontorta and Piceaengelmannii. Can J For Res 20:48–57. doi: 10.1139/x90-008

Ryan MG (1995) Foliar Maintenance Respiration of Sub-Alpine and Boreal Trees and Shrubs in Relation to Nitrogen-Content. Plant Cell Environ 18:765–772. doi: 10.1111/j.1365-3040.1995.tb00579.x

Ryan PPR, Delhaize E, Jones DDL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:527–560. doi: 10.1146/annurev.arplant.52.1.527

Saffell BJ, Meinzer FC, Woodruff DR, et al. (2014) Seasonal carbohydrate dynamics and growth in Douglas-fir trees experiencing chronic, fungal-mediated reduction in functional leaf area. Tree Physiol 34:218–228. doi: 10.1093/treephys/tpu002

Sakai A (1962) Studies on the Frost-Hardiness of Woody Plants I. The causal relation between sugar content and frost-hardiness. Contrib from Inst Low Temp Sci

Sala A (2009) Lack of direct evidence for the carbon-starvation hypothesis to explain drought-induced mortality in trees. Proc Natl Acad Sci U S A 106:E68. doi: 10.1073/pnas.0904580106

Sala A, Piper FI, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol 186:274–281. doi: 10.1111/j.1469-8137.2009.03167.x

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiol 32:764–775. doi: 10.1093/treephys/tpr143

Sardans J, Peñuelas J, Ogaya R (2008a) Drought-Induced Changes in C and N Stoichiometry in a Quercus ilex Mediterranean Forest. For Sci 54:513–522.

Sardans J, Peñuelas J, Ogaya R (2008b) Drought's impact on Ca, Fe, Mg, Mo and S concentration and accumulation patterns in the plants and soil of a Mediterranean evergreen Quercus ilex forest. Biogeochemistry 87:49–69. doi: 10.1007/s10533-007-9167-2

Scartazza A, Moscatello S, Matteucci G, et al. (2013) Seasonal and inter-annual dynamics of growth, non-structural carbohydrates and C stable isotopes in a Mediterranean beech forest. Tree Physiol 33:730–742. doi: 10.1093/treephys/tpt045

Schulze E-D (1989) Air Pollution and Forest Decline in a Spruce (Picea abies) Forest. Science 244:776–783. doi: 10.1126/science.244.4906.776

Schulze W, Gleixner G, Kaiser K, et al. (2005) A proteomic fingerprint of dissolved organic carbon and of soil particles. Oecologia 142:335–343. doi: 10.1007/s00442-004-1698-9

Sevanto S, McDowell NG, Dickman LT, et al. (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell Environ 37:153–161. doi: 10.1111/pce.12141

Silva DE (2010) Ecologie du hêtre (Fagus sylvatica L.) en marge sud-ouest de son aire de distribution. Université Henri Poincaré, Nancy

Sinclair W, Hudler G (1988) Tree declines: four concepts of causality. J Arboric 14:29–35.

Sparks JP, Black RA (1999) Regulation of water loss in populations of Populus trichocarpa: the role of stomatal control in preventing xylem cavitation. Tree Physiol 19:453–459. doi: 10.1093/treephys/19.7.453

Sperry JS (2000) Hydraulic constraints on plant gas exchange. Agric For Meteorol 104:13–23. doi: http://dx.doi.org/10.1016/S0168-1923(00)00144-1

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell Environ 11:35–40. doi: 10.1111/j.1365-3040.1988.tb01774.x

Spicer R (2014) Symplasmic networks in secondary vascular tissues: parenchyma distribution and activity supporting long-distance transport. J Exp Bot 65:1829–1848. doi: 10.1093/jxb/ert459

Spicer R, Holbrook MN (2007) Parenchyma cell respiration and survival in secondary xylem: Does mlanetabolic activity decline with cell age? Plant, Cell Environ 30:934–943. doi: 10.1111/j.1365-3040.2007.01677.x

Tamm CO, Pettersson A (1969) Studies on nitrogen mobilisation in forest soils. Stud For Suec 75:

Teissier du Cros E, Le Tacon F, Nepveu G, et al. (1981) Le hêtre, Quae. INRA, Paris

Tissier J, Lambs L, Peltier J-P, Marigo G (2004) Relationships between hydraulic traits and habitat preference for six Acer species occurring in the French Alps. Ann For Sci 61:81–86.

Tomlinson GHI (1983) Air pollutants and forest decline. Environ Sci Technol 17:246A–256A.

Torelli N, Čufar K, Robic D (1986) Some wood anatomical, physiological and silvicultural aspects of silver fir dieback in Slovenia (NW Yugoslavia). Iawa Bull 7:343–350.

Tromp J (1983) Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. Plant Soil 71:401–413. doi: 10.1007/BF02182682

Tyree MT, Sperry JS (1989) Vulnerability of Xylem to Cavitation and Embolism. Annu Rev Plant Biol 40:19–36.

Urli M, Porté AJ, Cochard H, et al. (2013) Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol 33:672–683. doi: 10.1093/treephys/tpt030

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. doi: http://dx.doi.org/10.1016/0038-0717(87)90052-6

Vennetier M (2012) Changement climatique et dépérissements forestiers. Chang Clim Modif For. pp 50–60

Vitasse Y, Delzon S, Dufrêne É, et al. (2009) Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? Agric For Meteorol 149:735–744. doi: 10.1016/j.agrformet.2008.10.019

Vogt KA, Vogt DJ, Palmiotto PA, et al. (1995) Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant Soil 187:159–219. doi: 10.1007/BF00017088

Wang Q, Iio A, Tenhunen J, Kakubari Y (2008) Annual and seasonal variations in photosynthetic capacity of Fagus crenata along an elevation gradient in the Naeba Mountains, Japan. Tree Physiol 28:277–285. doi: 10.1093/treephys/28.2.277

Wardle DA (1998) A comparative assessment of factors which influencemicrobial biomass carbon and nitrogen levels in soil. Biol Rev Camb Philos Soc 67:321–358. doi: 10.1111/j.1469-185X.1992.tb00728.x

Waring RH (1987) Characteristics of Trees Predisposed to Die. Bioscience 37:569–574.

Weber JA, Gates DM (1990) Gas exchange in Quercus rubra (northern red oak) during a drought: analysis of relations among photosynthesis, transpiration, and leaf conductance. Tree Physiol 7:215–225. doi: 10.1093/treephys/7.1-2-3-4.215

Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154:327–338. doi: 10.1007/s00442-007-0804-1

Wheeler JK, Huggett BA, Tofte AN, et al. (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. Plant Cell Environ 36:1938–1949. doi: 10.1111/pce.12139

Wildhagen H, Dürr J, Ehlting B, Rennenberg H (2010) Seasonal nitrogen cycling in the bark of field-grown Grey poplar is correlated with meteorological factors and gene expression of bark storage proteins. Tree Physiol 30:1096–1110. doi: 10.1093/treephys/tpq018

Wiley E, Helliker B (2012) A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. New Phytol 195:285–289. doi: 10.1111/j.1469-8137.2012.04180.x

Wiley E, Huepenbecker S, Casper BB, Helliker BR (2013) The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favour of storage? Tree Physiol 33:1216–1228. doi: 10.1093/treephys/tpt093

Van der Willigen C, Pammenter NW (1998) Relationship between growth and xylem hydraulic characteristics of clones of Eucalyptus spp. at contrasting sites. Tree Physiol 18:595–600. doi: 10.1093/treephys/18.8-9.595

Wortemann R, Herbette S, Barigah TS, et al. (2011) Genotypic variability and phenotypic plasticity of cavitation resistance in Fagus sylvatica L. across Europe. Tree Physiol 31:1175–1182.

Zak JC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: A quantitative approach. Soil Biol Biochem 26:1101–1108. doi: http://dx.doi.org/10.1016/0038-0717(94)90131-7

Zang U, Goisser M, Grams TEE, et al. (2014) Fate of recently fixed carbon in European beech (Fagus sylvatica) saplings during drought and subsequent recovery. Tree Physiol 34:29–38. doi: 10.1093/treephys/tpt110

El Zein R, Bréda N, Gérant D, et al. (2011a) Nitrogen sources for current-year shoot growth in 50-year-old sessile oak trees: an in situ 15N labeling approach. Tree Physiol 31:1390–1400.

El Zein R, Maillard P, Bréda N, et al. (2011b) Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. Tree Physiol 31:843–854. doi: 10.1093/treephys/tpr074

Zeppel MJB, Adams HD, Anderegg WRL (2011) Mechanistic causes of tree drought mortality: recent results, unresolved questions and future research needs. New Phytol 192:800–803.

Zhao J, Hartmann H, Trumbore S, et al. (2013) High temperature causes negative whole-plant carbon balance under mild drought. New Phytol 200:330–339. doi: 10.1111/nph.12400

Zimmermann MH (1957) Translocation of Organic Substances in Trees. I. The Nature of the Sugars in the Sieve Tube Exudate of Trees. Plant Physiol 32:288–291.

Zweifel R, Zimmermann L, Zeugin F, Newbery DM (2006) Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. J Exp Bot 57:1445–1459. doi: 10.1093/jxb/erj125

Abstract

The cases of forest decline reported in the literature have strongly increased during the last decade. This increase seems at least in part attributable to global change. However, the ecophysiological mechanisms underlying tree declines remain poorly understood. Two main hypotheses initially emerged from the recent literature: a deficit of carbon reserve compounds, or a failure of the hydraulic system of the tree. These hypotheses are not mutually exclusive, nor exhaustive: multiple couplings exist between the carbon and hydraulic functionings, and the internal nitrogen cycle of the trees could also be involved in tree decline. The main objective of this work was to document the ecophysiological mechanisms underlying a case of mature beech (*Fagus sylvatica* L.) drought-related decline in the Fontainebleau state forest. The decline was studied under non extreme climatic conditions. Therefore, we documented maily after-effects of past stresses. In this thesis, we developed an approach integrating the hydraulic, carbon and nitrogen aspects of functioning of the tree-soil system. The transfer of carbon compounds from tree roots to soil via the rhizodeposition process was also taken into account. Under non-stressful conditions, the functioning of healthy and declining trees is similar at the organ level, apart from a decreased stem radial growth in declining trees. In the trunk, radial growth thus appears to be the "adjustment variable" of declining trees in response to a lower whole-tree carbon assimilation due to their severely thinned crowns. Our results show that after a moderate hydric constraint, declining trees show a carbon reserve deficit in some organs compared to healthy trees. This deficit is compensated after a favourable year. A high resilience of carbon reserve concentrations could explain the ability of declining trees to survive for several years with a strongly reduced whole-tree leaf area. Healthy and declining trees presented different parenchyma ray proportions and structures in the stem xylem. Therefore, it could be interesting to study functional anatomical features in the future. Besides, tree rings characteristics could offer an insightful retrospective view of the history of this decline. Furthermore, an ongoing study on the structure of the microbial communities in the rhizosphere of healthy and declining trees will improve our knowledge about the impact of tree decline on the tree-soil interactions.

Key words: tree decline, *Fagus sylvatica*, hydraulic functioning, carbon functioning, internal nitrogen cycling, tree-soil interface

Résumé

Les dépérissements forestiers rapportés dans la littérature ont fortement augmenté durant la dernière décennie. Si cette augmentation semble attribuable au moins en partie aux changements globaux, les mécanismes écophysiologiques sous jacents restent encore mal connus. La littérature sur ce sujet a initialement permis de dégager deux principales hypothèses sur les mécanismes fonctionnels à l'œuvre lors d'un dépérissement : un manque de réserves carbonées et un dysfonctionnement du système hydraulique. Ces deux hypothèses ne sont pas mutuellement exclusives, ni exhaustives : les fonctionnements carboné et hydraulique interagissent à de multiples niveaux, et une implication du cycle interne de l'azote n'est pas à exclure. L'objectif principal de cette thèse est de documenter les mécanismes écophysiologiques à l'œuvre dans un dépérissement de hêtres (*Fagus sylvatica* L.) matures en forêt de Fontainebleau, lié principalement à des sécheresses répétées. Le dépérissement a ici été étudié en dehors de fortes contraintes climatiques. Ce sont donc majoritairement des arrières-effets de stress passés qui ont été renseignés. Au cours de ce travail, une approche intégrant des aspects hydraulique, carboné et azoté du fonctionnement du système arbre-sol a été adoptée afin de développer une vision intégrée de l'impact du dépérissement sur les grandes fonctions de l'arbre. Le transfert de composés de l'arbre vers le sol via la rhizodéposition a également été pris en compte. En dehors de périodes de stress, le fonctionnement des arbres sains et dépérissants à l'échelle de l'organe est similaire, à l'exception d'une diminution de la croissance radiale du tronc chez les arbres dépérissants. Au niveau du tronc, la croissance radiale apparait donc comme la variable d'ajustement des arbres dépérissants à une assimilation de carbone diminuée du fait de la détérioration de l'état de santé du houppier. Suite à une contrainte hydrique modérée, les arbres dépérissants présentent un déficit en réserves carbonées par rapport aux arbres sains dans certains organes. Ce déficit se trouve compensé après une année favorable. Une forte résilience des concentrations en réserves carbonées pourrait expliquer la capacité des arbres dépérissants à survivre de nombreuses années avec un houppier très endommagé. Des différences de proportion et de structure des rayons ligneux détectées entre les arbres sains et dépérissants laissent entrevoir l'intérêt qu'aurait dans le futur l'étude de caractéristiques anatomiques fonctionnelles dans le xylème. De plus, une approche rétrospective grâce aux cernes permettrait d'affiner notre compréhension de la mise en place du dépérissement à long terme. Par ailleurs, une étude actuellement en cours sur la structure des communautés microbiennes de la rhizosphère devrait permettre de compléter notre connaissance de l'impact du dépérissement sur les relations arbre-sol.

Mots-clef : dépérissement, *Fagus sylvatica*, fonctionnement hydraulique, fonctionnement carboné, cycle interne de l'azote, interface arbre-sol